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# Crop Response to Low-Dose Dicamba

Mason Castner
*University of Arkansas, Fayetteville*

Crop Response to Low-Dose Dicamba

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Mason Castner
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_____
Jason Norsworthy Ph.D.
Thesis Director

_____
Tom Barber Ph.D.
Committee Member

_____
Edward Gbur Ph.D.
Committee Member

_____
Trenton Roberts Ph.D.
Committee Member

## ABSTRACT

The introduction of dicamba-resistant (DR) soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.) followed by a label allowing over-the-top applications of dicamba in the 2017 provided growers an additional option for broadleaf weed control. Because non-DR soybean is sensitive to low concentrations of dicamba, postemergence dicamba applications present growers choosing to plant non-DR soybean with concerns of damage from off-target movement through physical drift and volatilization of the herbicide as well as tank-contamination from prior dicamba application. Consequences of low-dose dicamba exposure on sensitive vegetative and reproductive soybean have been well researched, but little is known regarding the interaction between low-dose dicamba exposure and commonly applied contact herbicides. There is also limited research assessing repercussions caused by dicamba exposure during reproductive development in corn (*Zea mays* L.) and rice (*Oryza sativa* L.), two common cereal crops in Arkansas in proximity to soybean and cotton. Experiments included evaluating the potential for dicamba to elicit a hormetic response to sensitive soybean, determining if contact herbicides exacerbated off-target dicamba symptomology, and evaluating potential for low-dose dicamba exposure on reproductive corn and rice to reduce grain yield. Low doses of dicamba did not improve soybean grain yield, which indicates a hormetic response is unlikely. Contact herbicides such as glufosinate and acifluorfen applied in close proximity to a low dose of dicamba increased visible auxin symptomology to soybean at 21 and 28 days after treatment (DAT) compared to dicamba alone, but no effect on grain yield occurred. Soybean was injured more when dicamba exposure followed a glufosinate application than when dicamba preceded glufosinate or was applied in a mixture with glufosinate, with yield reductions resulting when the contact herbicide was applied 7 to 10 days after a V3 dicamba exposure. Visible injury to corn

from dicamba was negligible (0%), but rice treated with dicamba at 56 and 560 g ae $ha^{-1}$ demonstrated auxin symptomology which led to reductions in grain yield. Corn grain yield could only be reduced from dicamba at 560 g ae $ha^{-1}$. In conclusion, dicamba tank-contamination and possibly off-target movement pose a greater threat to sensitive soybean regarding visible symptomology and yield as opposed to corn and rice, and it is unlikely that soybean exposure to low-dose dicamba will improve grain yields.

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# CHAPTER 1

## REVIEW OF LITERATURE

Currently, soybean [*Glycine max* (L.) Merr.] is the most dominant oilseed in the United States (US), accounting for roughly 90% of the US total oilseed production. Originally, European immigrants utilized soybean for forage production following its initial introduction during the 18$^{th}$ century. However, large-scale grain production eventually overcame forage production by the 1940s (Gibson and Benson 2005). Despite adoption of large-scale soybean production in the 20$^{th}$ century, soybean falls second to corn (*Zea mays* L.) in total area planted; 36.2 million hectares were planted in 2017, which is approximately 34% of the soybean market worldwide (USDA-NASS 2018). The Midwest is the largest soybean-producing region in the US, accompanied by several adjacent states. Along with the Midwest, states in the Midsouthern US occupying the Mississippi Delta region have many hectares dedicated to soybean production, with Arkansas ranking as the 9$^{th}$ largest producer in the US.

Glyphosate-resistant (GR) soybean was first introduced to the market in 1996, allowing for postemergence (POST) applications of glyphosate (N-phosphonomethyl glycine) for nonselective, broad-spectrum weed control in-crop (Dill 2005). Glyphosate was appealing to growers as it was economical and provided effective control over weed populations resistant to acetolactate synthase (ALS)- and protoporphyrinogen oxidase (PPO)-inhibitors (Norsworthy et al. 2008). However, lack of crop and herbicide rotation ultimately allowed prolific weeds such as Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] to evolve resistance to glyphosate over time. To combat ALS, PPO, and glyphosate-resistant weeds and further US soybean production, dicamba-, glufosinate-, 2,4-D-, and 4-hydroxphenyl dioxygenase-resistant soybean were introduced in the crop in the 21$^{st}$ century.

The incorporation of dicamba-resistant (DR) or Roundup Ready 2 Xtend (Xtend) soybean varieties in the state of Arkansas is concerning because dicamba has potential to volatilize and move off-target causing injury, yield loss, and additional herbicide applications to susceptible soybean varieties (Norsworthy et al. 2015). The risk associated with low-dose dicamba interactions with conventional and nonselective herbicides such as glyphosate and glufosinate are limited given the recent introduction of the Xtend technology. In lieu of widespread dicamba vapor drift events in 2017 (Bomgardner 2018), it is imperative to understand how these herbicides interact in multiple soybean production systems, and to determine any impact dicamba may have on reproductive monocotyledon (monocot) crops.

**Herbicide-Resistant Soybean.** Because of naturally evolved resistance to ALS-inhibiting herbicides in conventional soybean production, many growers began utilizing PPO-inhibiting herbicides as another alternative for weed control as efficacy among ALS-inhibiting herbicides began to decline. Because of the lack of herbicide rotation in conventional soybean production, various weed species began to evolve resistance to PPO-inhibiting herbicides (Owen and Zelaya 2005). However, following the introduction of GR soybean, glyphosate allowed growers to manage effectively problematic weed species. The rapid adoption of GR crops across the US and sole use of glyphosate led to the evolution of GR Palmer amaranth and other weed species. With the introduction of relatively new soybean technologies such as the Enlist, LibertyLink, LLGT27, and Xtend traits, producers now have effective tools in minimizing production losses due to weed infestations (Ervin et al. 2010). These recent crop technologies have assisted in improving weed control, lowered costs of production, simplified weed management strategies, and reduced soil erosion by growers implementing conservation tillage practices (Duke and Powles 2008).

***Glyphosate-Resistant Soybean.*** Glyphosate kills plants by inhibiting 5-enolypyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme essential for production of aromatic amino acids. This enzyme, found in plants, bacteria, and fungi, is not present in animals. The unique site of action (SOA) of glyphosate along with its relatively low cost and low-risk chemical properties made it an appealing yet highly effective broad-spectrum herbicide (Atkinson 1985; Malik et al. 1989). For soybean to become resistant to POST applications of glyphosate, a gene from *Agrobacterium* encoding a glyphosate insensitive EPSPS was inserted into the crop. When inserted, the *Agrobacterium* gene altered the shape of the EPSPS enzyme, preventing glyphosate from binding to the active site (Delannay et al. 1995; Padgette et al. 1995).

***Glufosinate-Resistant Soybean.*** Glufosinate-ammonium (glufosinate) is the active ingredient in the contact herbicide Liberty (Bayer Crop Science, Research Triangle Park, NC) and was first registered in the US for use as a herbicide in 1993. Glufosinate functions by inhibiting glutamine synthetase (GS), which is an essential component of ammonia assimilation in most plant species, allowing for the conversion of glutamate and ammonia into glutamine (Devine et al. 1993). Because of GS being inhibited by glufosinate, a buildup of toxic ammonium along with the depletion of glutamine occurs leading to plant death (Bellinder et al. 1987). Similar to glyphosate, glufosinate is a POST-applied herbicide that provides broad-spectrum control of broadleaf and grass species (Ahrens 1994).

By incorporating the phosphinothricin N-acetyltransferase (PAT) gene from the bacteria *Streptomyces viridochomogenes* into soybean, glufosinate-resistant varieties tolerate POST applications of glufosinate (Droge et al. 1992). By allowing the use of another POST-applied, non-selective, broad-spectrum herbicide such as glufosinate, growers could effectively control

GR weed populations given the correct timing of applications (Culpepper et al. 2000; Norsworthy et al. 2008).

***Dicamba-Resistant Soybean.*** Dicamba is a synthetic hormone-based herbicide derived from the phytohormone indole-3-acetic acid (IAA) that leads to an overproduction of auxin in broadleaf plants. Phytohormones found in plants such as IAAs are responsible for plant growth and development, which regulate growth and development, cell division, cellular elongation, tropic responses, floral meristem differentiation, leaf initiation, senescence, apical dominance, and root formation (Grossmann 2009). Dicamba-resistant crops such as cotton and soybean tolerate POST applications of dicamba by metabolizing the herbicide with insertion of dicamba monooxygenase (DMO), which prevents accumulation of toxic levels in the crop (Behrens et al. 2007). The incorporation of DR crops allows producers flexibility with a new technology and POST SOA to assist in combatting resistant broadleaf weed populations, specifically multiple-resistant Palmer amaranth.

**Crop Response to Synthetic Auxin Herbicides.** Various analogues of IAA have been commercially produced since the 1940s such as 1-napthalene acetic acid (1-NAA) and the phenoxycarboxylic acids such as 2-methyl-4-chlorophenoxyacetic acid (MCPA), and 2,4-dichlorophenoxy acetic acid (2,4-D) (Cobb 1992; Sterling and Hall 1997; Grossmann 2003; Fedtke and Duke 2005). The analogues of IAA mentioned above, or synthetic auxins, control broadleaf weeds by mimicking auxins at a greater intensity for an extensive amount of time, resulting in plant death. However, when present at low concentrations, synthetic auxins may stimulate growth and developmental processes in plants (Dayan et al. 2009).

***Off-Target Movement of Dicamba.*** In-crop applications of dicamba are concerning for producers planting sensitive soybean due to the frequency of unfavorable weather conditions that

encourage volatility. Factors such as higher temperatures and low relative humidity contribute to the conversion of dicamba into the free-acid form, a gaseous state, which is responsible for the off-target movement of dicamba (Egan and Mortensen 2012; Mueller et al. 2013). Previously, labeled POST applications of dicamba have not been a concern when utilized in burndown applications or in wheat (*Triticum aestivum* L.) because of less favorable weather conditions at or surrounding applications.

The diglycolamine (DGA) and *N*, *N*-bis(3-aminopropyl)methylamine (BAPMA) salt of dicamba are significantly less volatile formulations than the dimethylamine (DMA) salt of dicamba. However, the dimethylamine, DGA, and BAPMA salts of dicamba all rely on the attached amine groups to reduce volatility. The lower-volatile formulations such as DGA and BAPMA salts have a greater molecular weight, increased amine count, and different amine groups that may contribute to reduced dicamba volatility. However, the best indicator for volatility reducing potential are the amount of amine groups that can participate in hydrogen bonding (Sharkey et al. 2020). Despite having an advantage in volatility reduction capacity, DGA formulations may still be detected in the air at low concentrations following an application (Mueller et al. 2013). Although DGA formulations have less potential for secondary movement, both DGA and DMA formulations have the capability of moving off-target for at least 72 hours after application (Mueller et al. 2013; Bish et al 2019). Changes in wind direction after application are difficult to predict, making volatilized dicamba a risk for damage to soybean and other sensitive vegetation on all sides of an application.

***Crop Response to Dicamba.*** Common off-target dicamba symptomology on non-DR soybean varieties from dicamba particle or vapor drift include leaf cupping, stem and leaf epinasty, and cracked and swollen stems, as well as chlorosis and necrosis (Wax et al. 1969; Auch and Arnold

1978; Al-Khatib and Peterson 1999; Andersen et al. 2004; Kelley et al. 2005; Sciumbato et al. 2004). Comparable to 2,4-D, dicamba is another plant growth regulator or auxinic herbicide commonly used in burndown applications, POST applications for broadleaf control in monocot crops, as well as for POST applications in Xtend cropping systems. Dicamba shares similarities in both structure and SOA to 2,4-D, both of which stimulate tissue regeneration and callus induction (Dudits et al. 1975). It has been documented that dicamba regenerated more wheat plants compared to 2,4-D when applied in an induction medium (Papenfuss and Carman 1987). However, additional studies have shown that there are no differences in wheat callus formation between 2,4-D and dicamba (Redway et al. 1990). Concentrations of 0.02 to 0.1 mg $L^{-1}$ of dicamba in the induction medium provided the same results with similar concentrations of 2,4-D in previous findings (Bahieldin et al. 2000).

***Crop Response to 2,4-D.*** Regeneration of cultured cells is an essential tool for crop improvement; however, the regeneration of tissues in crops can vary depending upon the genotype, tissue type, media regime, and hormonal requirements. Previous research has indicated that regeneration of monocot plant tissues is more difficult than regeneration of dicot plant tissues *in vitro*. Plant growth regulators such as 2,4-D are widely used in burndown applications and for POST applications in monocot crops. 2,4-D is also widely used for wheat callus induction at extremely low concentrations because of its ability to stimulate plant growth (Scott et al. 1990). Strong evidence supporting the capacity of 2,4-D to induce somatic embryogenesis in plant cells was also confirmed in 1992 (Michalczuk et al. 1992). According to research conducted on the effects of 2,4-D on algae growth, concentrations of 2,4-D as minute as 0.02 mg $L^{-1}$ stimulated both the growth and photosynthetic rates of *S. quadricauda* (Wong 2000).

Despite soybean being highly sensitive to several synthetic auxin herbicides such as dicamba, 2,4-D results in little visible auxin symptomology to soybean as opposed to more sensitive crops such as cotton. Sublethal doses of 2,4-D applied at V3 and R2 caused almost no visible injury to soybean at 2 and 4 weeks after treatment (WAT) (Solomon and Bradley 2014).

***Hormetic Response.*** From a plant physiological standpoint, hormesis is defined as a positive plant growth response initiated from a low-dose exposure to a toxic agent that at high concentrations would pose inhibitory effects (Calabrese et al. 2007). Several studies have suggested the possibility that a hormetic response may occur from low-dose dicamba exposure on dicamba-sensitive soybean (Auch and Arnold 1978; Weidenhamer et al. 1989; Robinson et al. 2013). Synthetic auxins such as 2,4-D have been studied for decades evaluating its ability to stimulate plant growth at sub-lethal rates. Concentrations of 2,4-D at 0.5, 1, and 5 ppm significantly increased shoot elongation, and 0.5 and 1 ppm significantly increased leaf area index. Treatments of 2,4-D at 100 ppm or less increased number of seed per pod and hastened harvest maturity (Miller et al. 1962a,b). This instance of an observed hormetic response to 2,4-D on soybean suggests that increases in yield and yield components may be possible.

**Impact of Synthetic Auxin Herbicides on Reproductive Cereal Crops.** Synthetic auxin herbicides such as 2,4-D, dicamba, and MCPA were introduced to the market following World War II, revolutionizing weed management in cereal crops. Majority of synthetic auxin herbicides are only active in dicot species because of differences in metabolism and target-site sensitivity (Cobb 1992; Sterling and Hall 1997; Grossmann 2003). Following the introduction of synthetic auxins, multiple chemical families of auxin herbicides have been developed to enhance selectivity, with several herbicides demonstrating activity on monocot weed species in cereal crops. The current families of synthetic auxins include: phenoxycarboxylic acids, benzoic acids,

pyridinecarboxylic acids, aromatic carboxymethyl derivatives, and quinolinecarboxylic acids, all of which differ in activity depending upon species. Dicamba demonstrates greater activity on soybean in comparison to 2,4-D; however, 2,4-D is more active on cotton than dicamba (Al-Khatib and Peterson 1999; Johnson et al. 2012). The selectivity of synthetic auxin herbicides depends upon several components: physiological stage, plant tissue, and species (Grossmann 2009).

***Corn.*** Dicamba has been used in corn as a broadleaf herbicide for over 50 years; however, injury to corn may occur if POST applications are made beyond approximately V6 (Mingxia et al. 2011). One study examined five auxin herbicides: clopyralid, dicamba, 2,4-D, picloram, and fluroxypyr, on 3-leaf corn at 1, 2, and 4 kg $ha^{-1}$ (Vettakkorumakankav et al. 2002). Corn plants treated with dicamba at 1, 2, or 4 kg $ha^{-1}$ displayed auxin symptomology by varying degrees of lodging, and the highest rate demonstrated the greatest percentage of lodging. Dicamba at 4 kg $ha^{-1}$ reduced both brace roots and strength of the main root while clopyralid exhibited no pronounced effects on corn root development, and fluroxypyr completely disintegrated all roots.

***Rice.*** Rice and wheat producers have been utilizing 2,4-D in broadleaf weed control programs for decades. At certain growth stages, applications of 2,4-D have the potential to injure grass crops, specifically at or following reproductive development. Auxin symptomology caused by 2,4-D during vegetative growth stages in rice can be categorized leaf rolling, folding, and buggy-whipping. During rice reproductive stages, 2,4-D injury can be presented as malformations to panicles by preventing panicle emergence from the flag leaf or causing death to the panicle from a lack of development (Kaufman 1953).

Quinclorac belongs to the quinolinecarboxylic acid family of synthetic auxin herbicides, which was first introduced in 1992 to control select dicot weeds and propanil-resistant

barnyardgrass PRE and POST in rice (Talbert and Burgos 2007). Quinclorac is translocated throughout the plant, with the youngest vegetation functioning as the strongest sinks (Lamoureux and Rusness 1995; Grossman 1998). Quinclorac can be applied post-flood as a salvage option; however, the risk for rice injury increases resulting from reproductive development. Bond and Walker (2012) indicated that quinclorac applied during panicle initiation (PI) reduced rice yield compared to applications made at earlier growth stages, with differing results among the selected cultivars. Rice was more likely to be injured as a result of delayed quinclorac applications, which may suggest the potential for other synthetic auxins to elicit a similar response from sprayer contamination or a drift event during reproductive development.

**Herbicide Interactions.** Often, graminicides are mixed with broadleaf herbicides for broad-spectrum control. Postemergence herbicides may also be mixed with residual herbicides with differing SOAs to counteract selection pressure placed on POST herbicides (Hydrick and Shaw 1994; Webster and Shaw 1997; Lanclos et al. 2002). However, when mixed, differing SOAs may synergize or antagonize herbicidal activity on broadleaves or grasses. Mixtures of dicamba plus glufosinate have shown increased efficacy on Palmer amaranth compared with individual applications of dicamba or glufosinate alone; however, antagonism has been observed with this mixture (Botha et al. 2013). Other research has also shown an increase in weed control when dicamba and glufosinate are mixed (Chahal and Johnson 2012; Barnett et al. 2013). Because mixtures of dicamba and glufosinate elicit greater control of Palmer amaranth, tank contamination with sublethal rates of dicamba on susceptible soybean varieties may further exacerbate initial phytotoxicity caused by contact herbicides.

***Dicamba Contamination on GR Soybean.*** With the recent introduction of Roundup Ready Xtend soybean, it is likely that there will be cases of dicamba tank contamination on non-

dicamba-resistant cultivars that may ultimately cause injury and yield loss (Olszyk et al. 2015). It has been reported that susceptible soybean varieties have sustained yield losses up to 18% due to only a 5.6 g ae $ha^{-1}$ or 1% tank contamination of dicamba at a rate of 560 g ae $ha^{-1}$ (Derksen 1989; Griffin et al. 2013). Consistent with other findings, dicamba poses a much greater threat to reproductive soybean. According to the label, glyphosate may be applied until reproductive growth stage three (R3), which could potentially be detrimental to yield if there is tank contamination with dicamba (Anonymous 2012).

Field trials conducted in 2012 and 2014 further confirmed the effects of dicamba tank contamination on vegetative and reproductive soybean. A standard rate of glyphosate plus dicamba at 0, 0.75, 1.5, 3, 6, 15, 30, and 60 g ae $ha^{-1}$ were applied to simulate tank contamination at 0.5, 1, 2.5, 5, and 10% of field-use rates of dicamba. Correlating with other findings, classic dicamba symptomology (leaf cupping, epinasty, stunting, and necrosis) increases likewise with rate and days after application. Applications at both V3 and R1 resulted in significant yield loss, a reduction in seeds per plant, as well as a reduction in pods per plant despite V3 soybean initially showing greater injury than R1 treated soybean (Soltani et al. 2016).

***Synthetic Auxins and PPO-inhibiting Herbicides.*** Plant growth regulating herbicides such as 2,4-D and dicamba have been utilized for decades because of their effectiveness in controlling broadleaf weeds. In addition to broad-spectrum control, synthetic auxin herbicides are appealing to growers due to the lack of resistant weeds in comparison to other SOAs (Sterling and Hall 1997). When applied at a drift rate of 5.6 g ae $ha^{-1}$ (1% of labeled use rate) on vegetative growth stage three (V3) soybean, dicamba reduced yield up to 34%. Conversely, 2,4-D at 112 g ae $ha^{-1}$ (10% of labeled use rate) produced roughly an equivalent amount of injury (Andersen et al. 2004). Because sensitivity of soybean to dicamba is high compared to other dicotyledon (dicot)

crops such as cotton, POST applications of dicamba in-crop can present a major concern especially with the introduction of Xtend cropping systems. Research has shown that dicamba exposure during late vegetative or early reproductive growth stages can lead to devastating impacts on yield (Wax et al. 1969; Auch and Arnold 1978). In 2017, an application of a PPO-inhibiting herbicide appeared to exacerbate auxin symptoms on soybean following an off-target dicamba exposure near the time of the scheduled herbicide application (Castner et al. 2018). Dicamba at 5.6 g ae $ha^{-1}$ when applied with fomesafen at 330 g ai $ha^{-1}$ caused a 1090 kg $ha^{-1}$ soybean grain yield loss while dicamba alone accounted for a 370 kg $ha^{-1}$ reduction in grain yield (Kelley et al. 2005).

***PRE- and POST-applied PPO Injury.*** Protoporphyrinogen oxidase-inhibiting herbicides function by initiating the oxidation of protoporphyrinogen to protoporphyrin IX in the chlorophyll biosynthetic pathway. Eventually oxidation of protoporphyrinogen leads to lipid and cell membrane destruction from the formation of oxygen radicles membrane disruption and cell death (Becerril and Duke 1989a,b). Some PPO-inhibiting herbicides offer acceptable efficacy when used for both PRE and POST applications but can result in crop injury given unfavorable environmental conditions. PRE-applied PPO-inhibiting herbicides such as flumioxazin or sulfentrazone can lead to chlorosis, discoloration of veins, and reductions in internode length in some varieties of soybean if applied over-the-top or if rainfall splashes herbicide onto emerging plants (Swantek and Oliver 1996). POST-applied PPO-inhibiting symptomology caused by acifluorfen and lactofen often presented a mottled appearance of leaves with necrotic lesions on leaf surfaces and some leaf crinkling (Aulakh et al. 2016). Injury to soybean caused by labeled POST-applied PPO herbicides is often due to poor growing conditions, which hinders

metabolism of the herbicide and allows for the formation of oxygen radicles. Generally, injury from PPO herbicides is transient and does not affect yield (Legleiter and Johnson 2013).

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# CHAPTER 2

# IS THERE A HORMETIC RESPONSE OF SENSITIVE SOYBEAN TO DICAMBA

## ABSTRACT

Producers have the opportunity to apply the auxin herbicide dicamba over-the-top of dicamba-resistant (DR) soybean and cotton for broadleaf weed control. However, dicamba residue in sprayers may be difficult to remove, presenting applicators that treat non-DR soybean fields with additional challenges. As the adoption of DR crops or frequency of dicamba use increases in summer months, there is greater potential for volatilization of the herbicide, an additional risk for nearby non-DR soybean producers. There has been widespread speculation that a low-dose exposure of dicamba to sensitive soybean may elicit a positive response in yield or yield components of the crop. To test this hypothesis, experiments assessing the ability of dicamba to cause a hormetic response on a sensitive soybean variety were conducted in Fayetteville, Arkansas, in 2018 and 2019. Dicamba at 0.009, 0.014, 0.028, 0.056, and 0.112 g ae $ha^{-1}$ (1/60,000 to 1/5000 times the labeled rate) was applied to soybean at the V3 stage along with inclusion of a non-treated control. Similar treatments were applied to soybean at the R1 stage in a second experiment. There were 4 of a possible 60 treatment means across six yield component parameters in 2018 that had a positive response over the nontreated control, although no increase in yield was detected. In 2019, there were 11 of a possible 60 treatment means across the same yield component parameters that displayed a decrease and subsequently reduced soybean grain yield. The degree of auxin symptomology varied between site-years and did not consistently evoke compensatory effects with respect to a given dicamba concentration and yield component. Overall, there were no distinct trends that could lead to the conclusion that exposure of soybean to sub-lethal doses of dicamba increased grain yield.

**Nomenclature:** Dicamba; cotton, *Gossypium hirsutum* L.; soybean *Glycine max* (L.) Merr.

**Keywords:** Dicamba, hormetic, low-dose

## INTRODUCTION

The inability of glyphosate to control Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] as a result of resistance was first verified in 2005 in Macon County, Georgia (Sosnoskie and Culpepper 2005). Additionally, confirmation of glyphosate resistance was documented in Mississippi County, Arkansas shortly after the initial incident in Georgia (Norsworthy et al. 2008). Despite concerns for developing glyphosate-resistant (GR) weed populations, many growers continued to plant GR soybean on roughly 40% of all soybean acreage in the US in 2017 (USDA-NASS 2018). As the rapid evolution of GR weeds threatened conventional soybean production practices and placed increased selection pressure on recent technologies such as glufosinate-resistant soybean cultivars, the development of dicamba-resistant (DR) crops were considered as an alternative to control herbicide-resistant broadleaf weeds by allowing producers to apply dicamba postemergence (POST) in-crop.

Dicamba is a relatively economical herbicide that does not persist in the soil for extended periods or pose risks to humans or wildlife (Shaner 2014). Dicamba is a synthetic hormone-based herbicide that eventually leads to an overproduction of auxin in broadleaf plants eventually leading to plant death (Grossmann 2009). Because of the high-level of efficacy dicamba displays on Palmer amaranth, implementing DR crops stacked with GR traits in both cotton and soybean could offer producers exceptional control of otherwise problematic weeds with applications of dicamba alone or in various combinations with other sites of action (SOAs). Dicamba-resistant crops allow over-the-top applications of dicamba by metabolism of the herbicide through a bacterial gene, dicamba monooxygenase (DMO). Dicamba monooxygenase ultimately prevents the herbicide from reaching toxic levels in the plant. Additionally, DMO

encodes a Rieske nonheme monooxygenase that is capable of inactivating dicamba when expressed from nuclear or chloroplast genomes within transgenic plants (Behrens et al. 2007).

Weed control and management is an essential component in maximizing agronomic production systems; however, many previously effective chemical weed control options have since been restricted following the onset of weeds resistant to acetolactate synthase- (group 2), protoporphyrinogen oxidase- (group 14), and 5-enolpyruvel-shikimate-3-phosphate- (group 9) inhibiting herbicides in the southern US. Because of widespread resistance to those mentioned SOAs, dicamba for use in-crop may become an invaluable alternative when considering resistance management. When used as a burndown material or for POST applications in cereal crops such as corn (*Zea mays* L.), dicamba has been a valuable option. However, as dicamba applications have shifted towards in-crop applications in cotton and soybean, there has been a substantial increase in damage to sensitive soybean (Bish and Bradley 2017).

The diglycolamine (DGA) salt of dicamba is a considerably less volatile formulation than the dimethylamine (DMA) salt of dicamba, yet DGA formulations can still be detected in the air at low concentrations following an application. Findings from research suggests that dicamba volatilization is not only a factor of formulation but is also correlated with temperature and relative humidity (Mueller et al. 2013). Greater temperatures combined with low relative humidity have been observed to amplify the potential for dicamba to convert into its volatile free acid form, dicamba acid (Egan and Mortensen 2012; Mueller et al. 2013). Despite DGA having an advantage over DMA dicamba, with respect to volatility, both formulations have the potential to move off-target for at least 72 hours after an application (Mueller et al. 2013). Although DGA dicamba has demonstrated reduced volatility compared to prior formulations such as DMA dicamba, DGA dicamba still has the capability of volatilizing for an extended period. Extended

volatilization coupled with fluctuations in wind direction following an application make dicamba applications a liability to sensitive soybean as well as other sensitive vegetation in both the upwind and downwind direction.

Classic symptomology of reduced-rate dicamba exposure on non-DR soybean cultivars from physical or vapor drift consists of leaf cupping, stem and leaf epinasty, cracked and swollen stems, and chlorosis followed by necrosis (Wax et al. 1969; Auch and Arnold 1978; Al-Khatib and Peterson 1999; Andersen et al. 2004; Sciumbato et al. 2004; Kelley et al. 2005). Solomon and Bradley (2014) found that all injury symptoms from sublethal rates of dicamba applied to V3 and R2 soybean were contingent upon a specific auxin herbicide and rate combination and did not depend upon the growth stage that exposure occurred. Auxin injury to soybean increased as herbicide rate likewise increased. Injury to soybean from all low doses (0.028, 0.28, 2.8, and 28 g ae $ha^{-1}$) of dicamba applied at V3 had no impact on yield or seeds per pod; however, plants treated with dicamba at 2.8 and 28 g ae $ha^{-1}$ at R2 negatively influenced yield and reduced seeds per pod.

A plant's growth regulation, metabolism, morphogenesis, and physiological response to abiotic and biotic stimuli are facilitated by phytohormones, which interact with receptors (Grossmann 2009). There are several classifications of phytohormones, with auxins or indole-3-acetic acid (IAA) being some of the most essential hormones for plant development. Indole-3-acetic acids are ultimately responsible for regulating cell division, elongation, and tropic responses. In plants, auxins serve a major role in the developmental process of vascular tissues, floral meristem differentiation, leaf initiation, senescence, apical dominance, and root formation (Grossmann 2009).

Beginning production in the 1940s, laboratories were able to synthesize multiple analogues of IAA to create synthetic auxins with herbicidal properties. The resulting IAA derivatives include: 1-napthalene acetic acid and the phenoxycarboxylic acids such as 2-methyl-4-chlorophenoxyacetic acid and 2,4-dichlorophenoxy acetic acid (2,4-D) (Cobb 1992; Sterling and Hall 1997; Grossmann 2003; Fedtke and Duke 2009). The various synthetic analogues produce similar plant responses as IAA or naturally occurring phytohormones, with greater intensity for a greater period. Growth and development processes can be stimulated at low concentrations; however, at increasing concentrations, growth can be disrupted. Disruptions in growth caused by high levels of synthetic auxins are typically lethal to sensitive species (Dayan et al. 2009).

In plants, hormesis is defined as a positive growth response that is initiated from a low-dose exposure to an inherently toxic agent that would be lethal at high concentrations (Calabrese et al. 2007). The response elicited by a cell to a low-dose of a toxin is considered an adaptive compensatory process following an initial disruption in homeostasis (Mattson 2008). One study studies suggest that hormetic responses have the potential to reduce consequential effects from other environmental stresses, such as mild heat stress (Li et al. 2002).

Plant growth regulating herbicides such as 2,4-D have been studied for decades to evaluate stimulation of plant growth at sub-lethal concentrations. Research studying potential stimulatory effects of 2,4-D on soybean showed that growth regulators may improve several yield components (Miller et al. 1962a,b). Concentrations of 2,4-D at 0.5, 1, and 5 ppm significantly increased shoot elongation, while 0.5 and 1 ppm significantly increased leaf area index. Treatments of 2,4-D at 100 ppm and less increased number of seed per pod and hastened

crop maturity. Because of the success of 2,4-D in achieving hormetic responses, dicamba may potentially elicit crop responses similar to 2,4-D at extremely low doses.

Much research has been conducted to assess the influence of dicamba exposure on injury, yield, and yield components of sensitive soybean cultivars (Al-Khatib and Peterson 1999; Kelley et al. 2005; Egan and Mortensen 2012; Griffin et al. 2013; Robinson et al. 2013; Egan et al. 2014; Solomon and Bradley 2014; Soltani et al. 2016; Kniss 2018). However, there are limited studies addressing the potential for low concentrations of dicamba to encourage a hormetic response, or a positive response to an inherently phytotoxic herbicide. Following the commercial launch of dicamba for use in DR soybean and cotton in 2017, auxin symptomology was routinely observed on soybean. Hence, research was initiated to determine if there were any benefits to soybean yield from the widespread damage to sensitive soybean that could not be determined from a visible standpoint.

## MATERIALS AND METHODS

Experiments were conducted at the Milo J. Schult Agricultural Research and Education Center in Fayetteville, AR, on a Captina silt loam (Fine-silty, siliceous, active, mesic, Typic Fragiudults) composed of 14% sand, 76% silt, 10% clay, and 4.5% organic matter with a pH of 6.5 in 2018 and on a Leaf silt loam (Fine, mixed, active, thermic, Typic, Albaquults) with 34% sand, 53% silt, 13% clay, and 1.5% organic matter with a pH of 6.2 in 2019. Prior to planting, the field was prepared with a disk followed by a hipper, which formed 91 cm-wide beds for planting. An indeterminate, medium/tall, 4.7 maturity group glufosinate-resistant soybean cultivar (CZ 4748LL, BASF Corporation, Research Triangle Park, NC 27709) was planted on May 9, 2018, and May 15, 2019, at 360,000 seeds $ha^{-1}$. Trials consisted of plots measuring 7.6 m long by 3.6 m wide (four rows wide) with two adjacent nontreated rows on either side of the

plot. Alleys 3 m in width along with spray shields during application were implemented to minimize risk of dicamba movement onto adjacent plots via physical drift. Both trials received a broadcast preemergence application of flumioxazin (Valent Corporation, Walnut Creek, CA, 94596) at 70 g ai $ha^{-1}$ plus pyroxasulfone (BASF Corporation, Research Triangle Park, NC, 27709) at 90 g ai $ha^{-1}$ at planting. Additional herbicide applications of glufosinate (BASF Corporation, Research Triangle Park, NC, 27709) at 656 g ai $ha^{-1}$ and *S*-metolachlor (Syngenta, Greensboro, NC 27419) at 1,312 g ai $ha^{-1}$ were post-directed to row-middles to avoid foliar contact with soybean, and mechanical weeding and hand-weeding were incorporated until complete canopy coverage was achieved. In weeks that 2.5 cm of rainfall did not occur, trials were furrow-irrigated in 2018 and over-head irrigated in 2019 to simulate standard production practices and create a high yielding environment. The test site both years was fertilized based on soil test recommendations for soybean (Slaton et al. 2013) amended with plant essential nutrients based on soil test results acquired from each location.

The experiment was arranged as a single-factor randomized complete block design with four replications. Reduced rates of dicamba at 0, 0.0093, 0.014, 0.028, 0.056, and 0.112 g ae $ha^{-1}$ (1X rate being 560 g ae $ha^{-1}$) were applied to soybean at the V3 stage in the first experiment and R1 stage (initial bloom) in the second experiment. Applications were made with a $CO_2$-pressurized backpack sprayer calibrated to deliver 140 L $ha^{-1}$ at 4.8 km $hr^{-1}$, equipped with four AIXR 110015 flat fan nozzles (Teejet Technologies, Springfield, IL 62703). All applications were made to the center four rows of each plot to allow visual comparison with the nontreated outside two rows. Visible injury to soybean was rated by comparing the four treated rows to the adjacent two nontreated rows on both sides of each plot using the Behrens and Lueschen scale for dicamba injury scoring from 0 to 100%, which primarily focused on dicamba damage to

terminal growth (Behrens and Leuschen 1979) (Table 1). Soybean canopy height was also measured in cm on the same interval as dicamba injury following the V3 and R1 dicamba applications. Additionally, above-ground soybean biomass was collected from 10 plants per plot 28 days following the V3 and R1 applications from the two outside treated rows (to prevent having to account for harvested soybean plants when determining yield). The date of soybean maturity (R8) was recorded when 95% of the soybean pods within the treated area reached a mature color. Prior to harvest, 5 soybean plant samples were collected from the outside treated rows in 2018 and 15 samples in 2019 to assess yield components and individual plant yield. Subsequently, the two center rows of each plot were harvested using a small-plot combine (ALMACO, Nevada, IA 50201), and grain yield was determined by adjusting the harvested weights to 13% moisture. Additionally, a collected grain subsample from the combine for each plot was used to determine 100-seed weight of the grain.

**Statistical Analysis.** All normally distributed data for this experiment were analyzed with JMP Pro 14.3 (SAS Institute Inc., Cary, NC) using the Fit Model Function to produce analysis of variance tables to determine significance of fixed effects. Once significance was determined for all assessments, Dunnett's procedure ($\alpha = 0.05$) was used to distinguish if treatments differed from the nontreated control. For both years for each growth stage, site year was significant; therefore, experiments were analyzed separately by site year, and block (replication) was considered as a random effect. All injury rating data were not normal and assumed a beta distribution. Injury data were subjected to analysis of variance with SAS 9.4 using PROC GLIMMIX (Gbur et al. 2012) and means for injury data were separated using Fisher's protected LSD ($\alpha = 0.05$).

## RESULTS AND DISCUSSION

**Soybean Yield Components and Yield.** Response of soybean plants to dicamba differed between 2018 and 2019 (data not shown), thus yield components and yield were separated by site-year (Table 2). In 2018, there were 4 of a possible 60 treatment means for all soybean yield components and both growth stages that increased compared to the nontreated control (Table 3). That same year, no negative effects of dicamba on yield components were observed for either growth stage. Conversely, in 2019, dicamba elicited no positive effects regarding soybean yield components, but negatively affected 11 of the possible 60 treatment means recorded. A plausible explanation for a contrasting site-year difference may be due to an increased sample size in the 2019 growing season, with a smaller sample size in 2018 allowing for greater variability (Biau et al. 2008). Sample size increased from 5 plants per plot in 2018 to 15 plants per plot in 2019, a 3-fold increase. As sample size increased, the ability to capture a significant response with respect to soybean yield components and grain yield should likewise increase; however, a positive response was not observed in 2018.

In instances where soybean grain yield from sampled plants was less than plants collected from the nontreated control in 2019, compensatory effects of other yield components were not seen. In addition to one detected grain yield reduction (-3.64 g per plant from the nontreated control) from 2019 R1 treatments (dicamba at 0.028 g ae $ha^{-1}$), the random sampled R1 treated plants were not an appropriate indicator for the total grain yield (Table 3). Despite the numerical trend of decreasing grain yield for 2019 R1 treatments, the plot grain yield was comparable to the nontreated control (3.038 kg $ha^{-1}$) in all instances. With no treatments displaying a consistent increase in individual plant grain yield or plot grain yield, the likelihood of a hormetic response is negligible.

There have been multiple studies claiming a low-dose exposure of dicamba contributed to an increase in soybean grain yield (Auch and Arnold 1978; Robinson et al. 2013; Weidenhamer et al. 1989). However, when these and other trials were closely examined as part of a meta-analysis, there were no distinct trends with respect to increased yield and yield components resulting from soybean plots treated with varying low doses of dicamba (Kniss 2018). The lack of a positive effect on grain yield in this study is not surprising considering findings from reports by Kniss (2018) and others in the literature.

**Visible Injury.** Previous research has shown that dicamba rates of approximately 0.019 g ae $ha^{-1}$ (1/24,500X assuming 560 g ae $ha^{-1}$ = 1X) are enough to produce visible injury to soybean based on a single exposure at a carrier volume of 140 L $ha^{-1}$ (Jones et al. 2019), which these experiments replicate. By using a carrier volume of 140 L $ha^{-1}$, these studies reflect a single tank-contamination occurrence as opposed to physical drift or volatility. Research has shown that carrier volume heavily influences the extent of auxin symptomology seen on soybean, especially as the solution becomes more concentrated with dicamba due to a reduction in spray volume (Sperry et al. 2019). Furthermore, gaseous uptake of some air pollutants can be as high as 4,000 times greater than physical uptake through the cuticle (Kerstiens et al. 1992), causing disproportionally greater injury from gaseous dicamba than physical exposure to soybean (Norsworthy, unpublished data).

Auxin injury to soybean was affected by dicamba rate for the 2018 and 2019 site-years with the lack of injury at the R1 application timing in 2018 as an exception (Table 4). Many outside factors such as irrigation, fertility, and temperature have been documented to influence soybean yield following dicamba exposure (Al-Khatib and Peterson 1999; Auch and Arnold 1978; Kniss 2018), however; outside factors such as irrigation may also be responsible for

severity of herbicide symptomology. Uptake of herbicides, specifically dicamba, is known to increase as the availability of water likewise increases, especially in low-lowing areas of the field (Grantz et al. 2020). Less cumulative rainfall was observed following the R1 dicamba application timing in 2018, equating to approximately 1 cm from 0- to 14- DAT. Conversely, plots received approximately 3.4 cm during the same interval following dicamba exposure in 2019 (Figures 1 and 2), potentially leading to the greater amount of auxin symptomology observed. Uneven furrow irrigation in 2018 plots due to field topography combined with less rainfall could potentially result in reduced dicamba injury, and greater rainfall coupled with the uniformity of overhead irrigation for plots in 2019 may explain more apparent symptomology.

Dicamba in the spray solution sufficient enough to induce visible injury (0.028 to 0.112 g ae $ha^{-1}$ of dicamba) to soybean in 2018 caused no adverse effects on soybean yield components, with all significant yield components increased in comparison to the nontreated control (Tables 3, 4, and 5). A hormetic response was not observed regardless of the presence or absence of auxin symptomology.

Contrasting findings from 2018, soybean treated with low doses of dicamba at the V3 and R1 growth stages in 2019 only exhibited an adverse response, if any, even in the absence of visible injury, which subsequently led to a single reduction in grain yield per plant with an R1 application of dicamba at 0.028 g ae $ha^{-1}$ (Tables 3 and 5). However, it is important to note that minimal auxin injury was observed for R1 treatments in 2018 (0 to 2%, 14 and 28 DAT), whereas visible injury appeared to be more prevalent in R1 treatments in 2019 (0 to 40%, 14 and 28 DAT) (Table 5). Soybean has been documented to recover from up to 8.75 g ae $ha^{-1}$ dicamba exposure during early vegetative growth stages with minimal or no impact to grain yield (Barber et al. 2017; Egan et al. 2014), which does not explain why yield loss occurred from dicamba

rates lower than 8.75 g ae $ha^{-1}$ following V3 applications in 2019. However, observing reductions in soybean grain yield is common from dicamba exposures occurring during reproductive development (Griffin et al. 2013; Soltani et al. 2016).

**Height and Biomass.** Weidenhamer et al. (1989) concluded that soybean yield loss can be correlated to reductions in height, although visible auxin injury is generally more severe at comparable dicamba rates. However, several of the dicamba rates examined in studies conducted here caused little to no auxin symptomology, with no rate being indicative of a certain degree of yield loss. In 2018, no reductions or increases in soybean height were recorded at either V3 or R1 application timings at 14 or 28 DAT despite dicamba at 0.112 g ae $ha^{-1}$ causing up to 25% and 13% visible injury, respectively. Similar to 2018, applications of dicamba at 0.112 g ae $ha^{-1}$ in 2019 caused 30 and 11% visible injury at 14 and 28 DAT, respectively, but was not able to reduce soybean height (Table 5). Although no rates in these experiments significantly impacted soybean height, height reductions from dicamba rates approaching 560 g ae $ha^{-1}$ on sensitive soybean would be expected from both an early vegetative and reproductive dicamba exposure as soybean is actively growing. However, as dicamba exposure occurs when soybean begins to allocate a greater number of resources towards reproductive growth, the likelihood of a height reduction should likewise decrease.

Soybean biomass harvested 28 DAT was less indicative of a response to dicamba than visual injury (Table 5). In 2018 and 2019, the majority of soybean biomass for all dicamba rates were numerically greater than the nontreated control ranging from 1- to 25- percentage points across both site-years and application timings, with no rate or timing being indicative of a positive growth response. The lack of significant increases in soybean biomass led to the conclusion that a hormetic effect was not observed.

**Practical Implications.** Off-target movement of dicamba via physical or vapor drift has proven to be a difficult challenge for non-DR soybean producers in the United States, specifically in the Midsouthern region. In geographies such as or similar to the Midsouth, frequent off-target exposures of dicamba leads to symptomology on non-DR soybean due to sensitivity of soybean to extremely low doses (Auch and Arnold 1978; Egan et al. 2014; Jones et al. 2019; Solomon and Bradley 2014). However, few studies suggest the potential for soybean to elicit a hormetic response from low-dose exposure of dicamba, such as increases in grain yield or other yield determining factors (Auch and Arnold 1978; Robinson et al. 2013; Weidenhamer et al. 1989). The purpose of the studies conducted in Fayetteville, Arkansas, were aimed to determine if there were any benefits from low-dose dicamba exposure onto sensitive soybean, given that exposure has become a common occurrence in Arkansas following the launch of dicamba products labeled for POST applications.

Findings from this study show that a single low-dose, tank-contamination rate of dicamba applied at an early vegetative or reproductive growth stage is unlikely to induce a hormetic response for grain yield although some improved yield components may be observed such as the number of branches, pods per branch, or seeds per branch. For every treatment mean that indicated an increase in a yield component for 2018, there is almost a three-fold decrease in treatment means in 2019. Reductions in yield components and plant grain yield at the R1 application timings in 2019 may be a function of the samples collected considering that soybean typically does not respond negatively to a single exposure to dicamba to the extent seen here at the rates evaluated (Al-Khatib and Peterson 1999; Auch and Arnold 1978; Kniss 2018). The likelihood for non-DR soybean producers to experience an increase in grain yield from off-target dicamba exposure is negligible and does not appear to correlate with any specific dicamba rate

tested in this or in other research (Kniss 2018). Dicamba rates applied in this study pose little, if any, potential to consistently reduce single plant grain yield or other yield components from an exposure during a reproductive growth. Overall, based on a single extremely low-dose dicamba off-target or tank-contamination event where slight auxin symptomology is observed, there is no evidence that the herbicide improves soybean grain yield under the conditions evaluated within this trial.

To support the findings in this research, more trials need to be conducted that encompass a wide range of sensitive soybean cultivars and environmental conditions to ensure that the potential for a hormetic response of sensitive soybean to dicamba does not exist. Additionally, to rule out the possibility of hormesis, other auxinic herbicides such as 2,4-D could be evaluated using similar parameters as well. However, the off-target issues relating to dicamba in the Midsouth are not limited to a single exposure and are often a result of multiple low-dose exposures, which is another variable to consider when attempting to understand injury related to off-target movement and capturing a hormetic response.

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## TABLES

Table 1. Behrens and Leuschen (1979) soybean dicamba injury index.

| Rating | Description |
|---|---|
| 0 | No effect, plant normal |
| 10 | Slight crinkle of leaflets of terminal leaf |
| 20 | Cupping of terminal leaflets, slight crinkle of leaflets of second leaf, growth rate normal |
| 30 | Leaflets of two terminal leaves cupped, expansion of terminal leaf suppressed slightly |
| 40 | Malformation and growth suppression of two terminal leaves, terminal leaf size less than one-half that of control |
| 50 | No expansion of terminal leaf, second leaf size one-half or less that of control |
| 60 | Slight terminal growth, vigorous, malformed axillary shoot growth developing |
| 70 | Terminal bud dead, substantial, strongly malformed, axillary shoot growth |
| 80 | Limited axillary shoot growth, leaves present at time of treatment chlorotic with slight necrosis |
| 90 | Plant dying, leaves mostly necrotic |
| 100 | Plant dead |

Table 2. The p-values from analysis of variance for soybean yield components: Pods and seeds on the main stem, number of branches, pods and seeds on branches, 100-seed weight, and grain yield from 2018 and 2019 in Fayetteville, AR.[a]

| Source | Site-year | App.[b] Timing | Pods/Main | Seeds/Main | Branches | Pods/ Branches | Seeds/ Branches | 100-seed weight | Grain yield |
|---|---|---|---|---|---|---|---|---|---|
| | | | ----------Prob. > F---------- | | | | | | |
| Dicamba rate | 2018 | V3 | 0.836 | 0.454 | 0.065 | 0.176 | 0.191 | 0.129 | 0.887 |
| Dicamba rate | | R1 | 0.251 | 0.053 | 0.006* | 0.027* | 0.046* | 0.549 | 0.493 |
| Dicamba rate | 2019 | V3 | 0.031* | 0.008* | 0.240 | 0.123 | 0.150 | 0.534 | 0.483 |
| Dicamba rate | | R1 | 0.038* | 0.001* | 0.112 | 0.007* | 0.020* | 0.004* | 0.435 |

[a]P-values within columns denoted by asterisks indicate significance.
[b]Abbreviation: App, application.

Table 3. Soybean yield components (pods/main, seeds/main, branches, pods/branches, seeds/branches, and 100-seed weight), grain yield, and plot grain yield collected post-harvest analyzed separately by site-year and application timing of dicamba from 2018 and 2019 in Fayetteville, AR.[a]

| Site-year | Rate | App.[b] Timing | Yield components | | | | | | Grain yield[ce] | Plot grain yield[f] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pods/Main[d] | Seeds/Main[d] | Branches[d] | Pods/ Branches[d] | Seeds/ Branches[d] | 100-seed weight[e] | | |
| | | | % of nontreated | | | | | | | |
| 2018 | 0 | V3 | 100 (38) | 100 (95) | 100 (1) | 100 (4) | 100 (10) | 100 (17) | 100 (18) | 100 (2,960) |
| | 0.112 | | 103 | 98 | 146 | 214 | 194 | 99 | 106 | 98 |
| | 0.056 | | 111 | 114 | 125 | 110 | 105 | 97 | 109 | 109 |
| | 0.028 | | 111 | 107 | 92 | 156 | 138 | 97 | 106 | 110 |
| | 0.014 | | 102 | 105 | 108 | 106 | 98 | 95 | 99 | 109 |
| | 0.009 | | 104 | 116 | 196 | 200 | 197 | 103* | 127 | 105 |
| | 0 | R1 | 100 (39) | 100 (98) | 100 (1) | 100 (5) | 100 (12) | 100 (17) | 100 (20) | 100 (3,090) |
| | 0.112 | | 120 | 119 | 171 | 163 | 156 | 98 | 120 | 104 |
| | 0.056 | | 109 | 107 | 225* | 307* | 271* | 99 | 121 | 92 |
| | 0.028 | | 105 | 93 | 113 | 121 | 107 | 99 | 93 | 89 |
| | 0.014 | | 100 | 95 | 133 | 139 | 126 | 100 | 98 | 92 |
| | 0.009 | | 101 | 97 | 96 | 119 | 113 | 97 | 96 | 109 |
| 2019 | 0 | V3 | 100 (46) | 100 (88) | 100 (2) | 100 (14) | 100 (25) | 100 (15) | 100 (17) | 100 (3,560) |
| | 0.112 | | 88 | 87 | 98 | 74 | 77 | 95 | 80 | 93 |
| | 0.056 | | 92 | 88 | 86 | 63 | 63 | 94 | 77 | 106 |
| | 0.028 | | 88 | 88 | 85 | 84 | 93 | 95 | 85 | 81 |
| | 0.014 | | 83* | 78* | 76 | 65 | 67 | 94 | 71 | 88 |
| | 0.009 | | 87 | 85 | 99 | 93 | 94 | 95 | 83 | 93 |
| | 0 | R1 | 100 (29) | 100 (59) | 100 (2) | 100 (13) | 100 (26) | 100 (15) | 100 (13) | 100 (3,038) |
| | 0.112 | | 90 | 95 | 89 | 56* | 59* | 97 | 82 | 138 |

Table 3. Cont. Soybean yield components (pods/main, seeds/main, branches, pods/branches, seeds/branches, and 100-seed weight), grain yield, and plot grain yield collected post-harvest analyzed separately by site-year and application timing of dicamba from 2018 and 2019 in Fayetteville, AR.[a]

| Site-year | Rate | App.[b] Timing | Yield components | | | | | | Grain yield[ce] | Plot grain yield[f] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pods/Main[d] | Seeds/Main[d] | Branches[d] | Pods/ Branches[d] | Seeds/ Branches[d] | 100- seed weight[e] | | |
| | | | % of nontreated | | | | | | | |
| 2019 | 0.056 | R1 | 97 | 92 | 68 | 65 | 70 | 99 | 85 | 147 |
| | 0.028 | | 88* | 81* | 84 | 79 | 78 | 90* | 72* | 145 |
| | 0.014 | | 98 | 88* | 104 | 99 | 100 | 92* | 84 | 153 |
| | 0.009 | | 91 | 86* | 89 | 92 | 86 | 96 | 82 | 143 |

[a]Means denoted by asterisks within a column are different from the nontreated control according to a $\alpha=0.05$ when using Dunnett's procedure.

[b]Abbreviation: App., application.

[c]Grain yield of individual soybean plants calculated based on the total number of seeds per plant and 100-seed weight.

[d]Numbers in parenthesis within a column represent actual number of pods/main, seeds/main, branches, pods/branches, and seeds/branches of the nontreated control.

[e]Numbers in parenthesis within a column represent actual 100-seed weight of the nontreated control in grams.

[f]Numbers in parenthesis within a column represent actual grain yield of the harvested plot in kg $ha^{-1}$.

Table 4. The p-values from analysis of variance for soybean auxin injury, height, and biomass from 2018 and 2019 in Fayetteville, AR.[a]

| Source | Site-year | Application timing | Auxin injury | | Height | | Biomass |
|---|---|---|---|---|---|---|---|
| | | | 14 DAT[b] | 28 DAT[b] | 14 DAT[b] | 28 DAT[b] | |
| | | | ----------Prob. > F---------- | | | | |
| Dicamba rate | 2018 | V3 | <0.001* | <0.001* | 0.742 | 0.483 | 0.582 |
| Dicamba rate | | R1 | 0.895 | 0.552 | 0.344 | 0.445 | 0.037* |
| Dicamba rate | 2019 | V3 | <0.001* | <0.001* | 0.880 | 0.528 | 0.619 |
| Dicamba rate | | R1 | <0.001* | <0.001* | 0.800 | 0.034* | 0.674 |

[a]P-values within columns denoted by asterisks indicate significance.
[b]Abbreviation: DAT, days after treatment.

Table 5. Soybean auxin injury, height, and biomass collected pre-harvest and analyzed separately by site-year and application timing from 2018 and 2019 in Fayetteville, AR.

| | | | Auxin injury[bc] | | Height[ad] | | Biomass[e] |
|---|---|---|---|---|---|---|---|
| Site-year | Rate | Application timing | 14 DAT | 28 DAT | 14 DAT | 28 DAT | 28 DAT |
| | g ae ha$^{-1}$ | | ----------% of nontreated---------- | | | | |
| 2018 | 0 | V3 | 0 d | 0 b | 100 (34) | 100 (58) | 100 (80) |
| | 0.112 | | 25 a | 13 a | 100 | 95 | 119 |
| | 0.056 | | 9 b | 1 b | 98 | 100 | 108 |
| | 0.028 | | 5 c | 0 b | 96 | 97 | 104 |
| | 0.014 | | 0 d | 0 b | 101 | 89 | 114 |
| | 0.009 | | 0 d | 0 b | 102 | 100 | 107 |
| | 0 | R1 | 0 | 0 | 100 (65) | 100 (79) | 100 (118) |
| | 0.112 | | 0 | 2 | 99 | 95 | 101 |
| | 0.056 | | 0 | 1 | 100 | 99 | 103 |
| | 0.028 | | 0 | 0 | 105 | 101 | 86 |
| | 0.014 | | 0 | 0 | 96 | 94 | 102 |
| | 0.009 | | 0 | 0 | 102 | 103 | 108 |
| 2019 | 0 | V3 | 0 d | 0 b | 100 (35) | 100 (56) | 100 (160) |
| | 0.112 | | 30 a | 11 a | 95 | 91 | 122 |
| | 0.056 | | 9 b | 2 b | 102 | 102 | 125 |
| | 0.028 | | 5 c | 0 b | 95 | 96 | 116 |
| | 0.014 | | 0 d | 0 b | 102 | 99 | 98 |
| | 0.009 | | 0 d | 0 b | 98 | 98 | 103 |
| | 0 | R1 | 0 d | 0 d | 100 (57) | 100 (71) | 100 (176) |
| | 0.112 | | 35 a | 40 a | 100 | 91 | 92 |
| | 0.056 | | 16 b | 19 b | 100 | 101 | 94 |
| | 0.028 | | 11 c | 12 c | 104 | 97 | 104 |
| | 0.014 | | 2 d | 1 d | 105 | 101 | 105 |
| | 0.009 | | 0 d | 0 d | 100 | 103 | 115 |

[a]Means denoted by asterisks within a column are different than the nontreated control according to a $\alpha$=0.05 when using Dunnett's procedure.
[b]Abbreviation: DAT, days after treatment.
[c]Means were subjected to ANOVA including the nontreated control and means within a column followed by the same letter are not different according to Fisher's protected LSD ($\alpha$=0.05).
[d]Numbers in parenthesis within a column represent actual height of the nontreated control in centimeters.
[e]Numbers in parenthesis within a column represent actual biomass of the nontreated control in grams.

## FIGURES

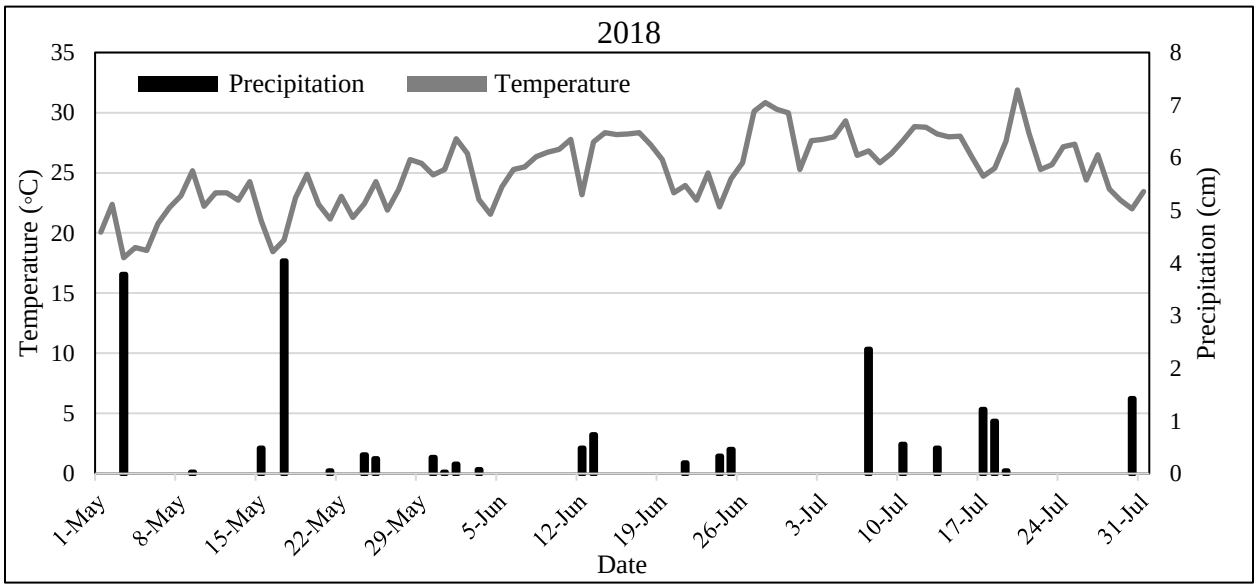


Figure 1. Precipitation and temperature history for 2018 in Fayetteville, AR from 1-May through 31-July.

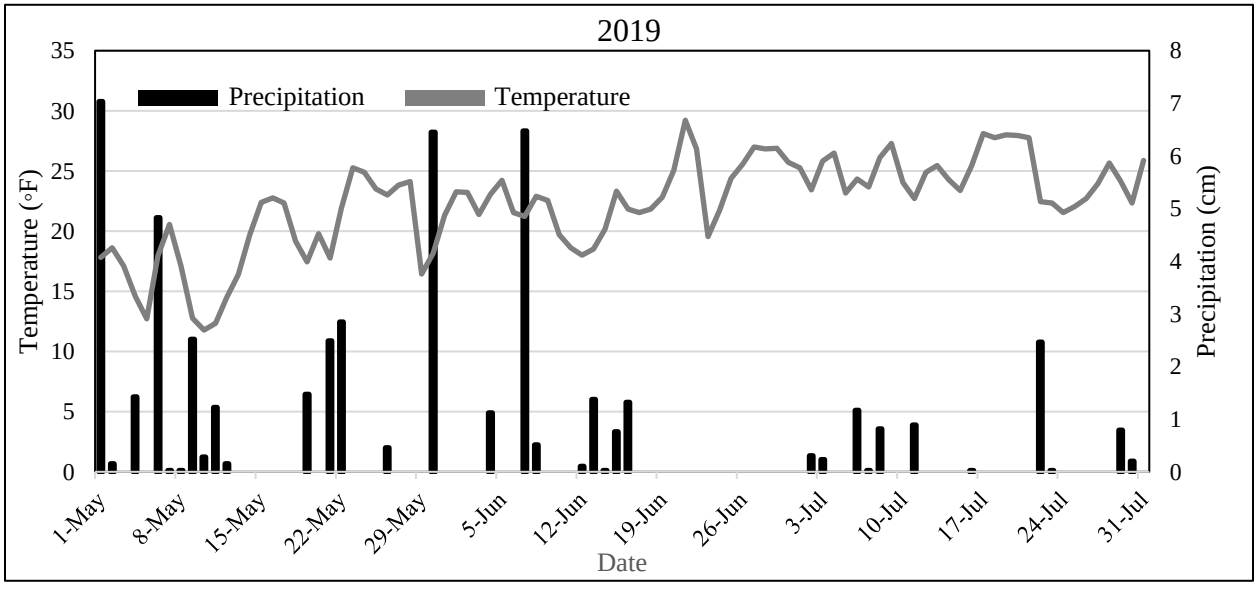


Figure 2. Precipitation and temperature history for 2019 in Fayetteville, AR from 1-May through 31-July.

# CHAPTER 3

# INTERACTION OF CONTACT HERBICIDES AND TIMING OF DICAMBA EXPOSURE ON SOYBEAN

## ABSTRACT

With recent advancements in crop technology, producers have been given the opportunity to effectively control problematic broadleaf weeds with dicamba applied postemergence in dicamba-resistant (DR) crops. Dicamba residue in sprayers may be difficult to remove and may interact with contact herbicides labeled for use in soybean. Without proper tank cleanout, applicators treating both DR and non-DR crops are at high risk for contaminating sensitive soybean cultivars. To evaluate both the consequences of dicamba tank contamination with contact herbicides and the impact that timing of dicamba exposure have on a glufosinate-resistant soybean cultivar relative to a contact herbicide application, experiments were conducted in Fayetteville, Arkansas, in 2018 and 2019. The experiments for tank contamination and for timing of dicamba exposure were set up as a two-factor and a single-factor randomized complete block design with four replications, respectively. For the first experiment, dicamba at 0, 0.056, 0.56, and 5.6 g ae $ha^{-1}$ was applied alone, with glufosinate, with acifluorfen, or with glufosinate plus acifluorfen to V3 soybean. For the second experiment, dicamba was applied at 2.8 g ae $ha^{-1}$ at VC, V1, V2, V3, and at 3, 7, and 10 days after a glufosinate application to V3 soybean (DATV3). Dicamba applied in combination with contact herbicides exacerbated visible auxin symptomology over dicamba alone at 21 and 28 days after treatment (DAT), while dicamba at 5.6 g ae $ha^{-1}$ reduced soybean height. Injury caused by dicamba mixtures with contact herbicides and height reductions averaged over dicamba rates did not reduce soybean grain yield. For the timing of the dicamba exposure experiment, soybean was injured more when dicamba exposure

followed a glufosinate application than when dicamba preceded glufosinate or was applied in a mixture with glufosinate, with yield reductions resulting from 7 and 10 DATV3 dicamba applications. Ultimately, dicamba exposure in the presence of contact herbicides resulted in increased auxin symptomology to soybean, which may be further intensified when dicamba exposure occurs following a contact herbicide application.

**Nomenclature:** Acifluorfen; dicamba; glufosinate; soybean, *Glycine max* (L.) Merr.

**Keywords:** Dicamba, tank contamination, timing of dicamba application, soybean

## INTRODUCTION

Producers frequently combine multiple herbicides into a single application to achieve greater weed control and to save application costs by reducing the number of trips across the field (Rhodes and Coble 1984; Jordan 1995; Zhang et al. 2005). Many broadleaf active herbicides can be mixed with graminicides for broad-spectrum control. Nonselective postemergence (POST)-applied herbicides such as glufosinate may be combined with herbicides that offer extended residual activity such as *S*-metolachlor. One advantage of combining herbicides with different sites of action (SOA) is that longevity of a single herbicide or SOA may be extended. This is important for producers facing resistance issues, especially as a tremendous amount of selection pressure can be placed on POST applications (Hydrick and Shaw 1994; Webster and Shaw 1997; Lanclos et al. 2002; Norsworthy et al. 2012).

Before mixing herbicides with different SOAs, it is important to consider the possibility that some combinations may demonstrate compatibility issues as well as antagonistic or synergistic interactions. Mixing systemic and contact herbicides such as dicamba and glufosinate have been documented to increase efficacy on Palmer amaranth (*Amaranthus palmeri* S. Watson) compared to an application of either herbicide alone (Chahal and Johnson 2012; Barnett et al. 2013). Despite some research showing increased control of Palmer amaranth with this mixture, antagonism has been observed on Palmer amaranth as well (Botha et al. 2013). Due to the potential for mixtures of dicamba and glufosinate to have increased control of Palmer amaranth than each herbicide alone, tank contamination with sublethal rates of dicamba on susceptible soybean cultivars may intensify initial transient phytotoxicity that is commonly associated with contact herbicides. Tank-contamination rates of dicamba may also increase the severity of visible auxin symptomology by providing additional plant stress. However,

glufosinate has been documented to antagonize the efficacy of clethodim on goosegrass [*Eleusine indica* (L.) Gaertn.] by disrupting cell membranes via accumulation of toxic levels of ammonia, which results in reduced translocation of clethodim (Burke et al. 2005). If soybean displays minor to moderate contact symptomology, which is common with protoporphyrinogen oxidase (PPO)-inhibiting herbicides, translocation of dicamba could be limited, thus reducing the degree of auxin symptomology.

Prior to the commercialization of glyphosate-resistant (GR) soybean, acetolactate synthase (ALS) and PPO-inhibiting herbicides were commonly utilized in POST applications for broadleaf weed control in soybean. With ALS and GR weed populations increasing as a result of extensive use of those SOAs, PPO herbicides became an essential chemical alternative for producers managing those resistant weed populations (Legleiter et al. 2009). Herbicides that function by inhibition of protoporphyrinogen oxidase oxidize protoporphyrinogen to protoporphyrin IX in the chlorophyll biosynthetic pathway. Oxidation of protoporphyrinogen leads to plant death by a buildup of intermediate materials that disrupt cell membranes (Becerril and Duke 1989a,b).

Several PPO-inhibiting herbicides are highly effective when used for both preemergence (PRE) and POST applications but may result in crop injury with unfavorable environmental conditions. Postemergence-applied PPO-inhibiting symptomology is often presented as necrotic lesions on trifoliates with minor leaf crinkling (Aulakh et al. 2016). Although contact herbicides such as glufosinate and acifluorfen do not share the same SOA, both initiate cell membrane disruption and therefore have the potential to reduce dicamba uptake and translocation, which may help to counteract reduced rates of dicamba in a tank contamination scenario. Contrary to documented antagonism between contact and systemic herbicides, experiments conducted by

Kelley et al. (2005) revealed that labeled POST-applied herbicides in soybean synergistically interacted with reduced rates of dicamba, with dicamba alone causing considerably more soybean injury than other common POST herbicides alone (≈40% increase) and resulted in an 18% increase in auxin injury when combined with fomesafen at the V3 growth stage.

With the introduction of the Roundup Ready 2 Xtend soybean technology, dicamba tank contamination is inevitable to non-dicamba-resistant (DR) cultivars especially with producers who utilize custom applicators (Soltani et al. 2016). Dicamba residues also have a high affinity to be deposited and sequestered into spray application equipment, specifically hoses and screens, creating risks for growers choosing to plant both (DR and dicamba-susceptible soybean cultivars (Cundiff et al. 2017). Commonly applied POST herbicides such as glyphosate and glufosinate act as effective tank cleaners due to the combined adjuvants within them, which often result in dicamba injury following a POST application from dissolved residues placed back into solution (Cundiff et al. 2017). Dicamba susceptible soybean cultivars have sustained yield losses up to 18% from only 1% tank contamination rate of dicamba, assuming 560 g ae $ha^{-1}$ as the labeled rate (Griffin et al. 2013). Dicamba poses greater risk to reproductively growing soybean in comparison to vegetative soybean (Solomon and Bradley 2014); however, research has shown yield loss associated with V3 dicamba exposure when combined with other POST herbicides (Kelley et al. 2005). According to the Roundup PowerMAX label, glyphosate may be applied until reproductive growth stage three (R3), which could potentially be detrimental to yield if tank contamination of dicamba occurred during reproductive development (Anonymous 2012). Reduced rates of dicamba (0, 0.75, 1.5, 3, 6, 15, 30, and 60 g ae $ha^{-1}$) applied in combination with glyphosate (1,260 g ae $ha^{-1}$) on both V3 and R1 soybean demonstrated significant injury and yield loss despite greater auxin symptomology on V3 applications (Soltani et al. 2016).

Synthetic auxin herbicides such as 2,4-D and dicamba have been utilized for decades because of their effectiveness in controlling broadleaf weeds (Egan and Mortensen 2012). In addition to broad-spectrum control, synthetic auxin herbicides are appealing to growers due to the lack of resistant weeds in comparison to other SOAs (Sterling and Hall 1997). When dicamba was applied to soybean at 5.6 g ae $ha^{-1}$ (1% of labeled use rate) at the V3 growth stage, grain yield was reduced up to 34%. Because sensitivity of soybean to dicamba is high in comparison to other dicotyledon (dicot) crops such as cotton (*Gossypium hirsutum* L.) (Egan et al. 2014), POST applications of dicamba in-crop can present a major concern especially with the introduction of Xtend cropping systems. Extensive research has documented that dicamba exposure during late vegetative or early reproductive growth stages can lead to soybean yield loss (Wax et al. 1969; Auch and Arnold1978; Scholtes et al. 2019).

Research has been conducted looking at the impact dicamba exposure has on injury, yield, and yield components of sensitive soybean cultivars; however, few have addressed whether commonly applied contact herbicides labeled in soybean interact with dicamba exposure via tank-contamination or volatilization, especially in geographies where contamination would be a concern. In 2017 with the launch and extensive use of dicamba in Xtend soybean production systems, dicamba-like symptoms were commonly observed on soybean, and the severity of the injury often appeared to be greater on non-DR soybean treated with a labeled, POST contact herbicide, such as PPO inhibitors or glufosinate (J.K. Norsworthy, personal communication). This observation led to research aimed at evaluating the impact dicamba tank-contamination or exposure could have on a sensitive soybean cultivar when applied with labeled contact herbicides or when soybean exposure to sublethal rates of dicamba occurs near the V3 timing of a glufosinate application.

## MATERIALS AND METHODS

**Common Methodology.** Experiments were conducted at the Milo J. Schult Agricultural Research and Education Center in Fayetteville, AR on a Captina silt loam (Fine-silty, siliceous, active, mesic, Typic Fragiudults) composed of 14% sand, 76% silt, 10% clay, and 4.5% organic matter with a pH of 6.5 in 2018, and a Leaf silt loam (Fine, mixed, active, thermic, Typic, Albaquults) with 34% sand, 53% silt, 13% clay, and 1.5% organic matter with a pH of 6.2 in 2019. Prior to planting, the field was prepared with a disk followed by a hipper, which formed 91 cm-wide beds for planting. An indeterminate, medium/tall, 4.7 maturity group glufosinate-resistant soybean cultivar (CZ 4748LL, BASF Corporation, Research Triangle Park, NC 27709) was planted on May 9, 2018, and May 15, 2019, at 360,000 seeds $ha^{-1}$. Trials consisted of plots measuring 7.6 m long by 1.8 m wide (two rows wide) with a nontreated row on either side of the plot. Alleys 3 m in width along with spray shields during application were implemented to minimize risk of dicamba movement onto adjacent plots via physical drift. Both trials received a broadcast PRE application of flumioxazin (Valent Corporation, Walnut Creek, CA 94596) at 70 g ai $ha^{-1}$ plus pyroxasulfone (BASF Corporation, Research Triangle Park, NC 27709) at 90 g ai $ha^{-1}$ at planting. Additional herbicide applications of glufosinate (BASF Corporation, Research Triangle Park, NC, 27709) at 656 g ai $ha^{-1}$ and *S*-metolachlor (Syngenta, Greensboro, NC 27419) at 1,312 g ai $ha^{-1}$ were post-directed to row-middles to avoid foliar contact with soybean, and mechanical weeding and hand-weeding were incorporated until complete canopy coverage was achieved. In weeks that 2.5 cm of rainfall did not occur, trials were furrow-irrigated in 2018 and over-head irrigated in 2019 to simulate standard production practices and create a high yielding environment. The test site both years was fertilized based on soil test recommendations for

soybean (Slaton et al. 2013) amended with plant essential nutrients based on soil test results acquired from each location.

Treatments were applied with a $CO_2$-pressurized backpack sprayer calibrated to deliver 140 L $ha^{-1}$ at 4.8 km $h^{-1}$ equipped with four AIXR 110015 flat fan nozzles (Teejet Technologies, Springfield, IL 62703). Visible injury to soybean was rated by comparing the two treated rows to the adjacent two non-treated rows on both sides of each plot using the Behrens and Lueschen scale for dicamba injury scoring from 0 to 100% at 21 and 28 DAT, which primarily focused on dicamba damage to terminal growth (Behrens and Leuschen 1979) (Table 1). Soybean canopy height was measured at three locations within each plot on the same interval as visible injury following the herbicide treatments. The date of soybean maturity was recorded when 95% of the soybean pods within the treated area reached a mature color. Subsequently, both rows of each plot were harvested using a small-plot combine (ALMACO, Nevada, IA 50201), and grain yield was determined by adjusting the harvested weights to 13% moisture. Additionally, a grain subsample was collected from the combine during harvest and later used to determine 100-seed weight.

***Dicamba Tank-Contamination.*** The experiment was arranged as a three-factor factorial randomized complete block design with four replications, with the first factor being site-year. The second factor represented the labeled rates of POST herbicides: glufosinate alone at 656 g ai $ha^{-1}$, acifluorfen (UPL, King of Prussia, PA 19406) alone at 560 g ai $ha^{-1}$, a glufosinate plus acifluorfen mixture at their respective labeled rates, or no additional herbicide. The third factor was four levels of simulated tank contamination rates of the Engenia (BASF Corporation, Research Triangle Park, NC, 27709) formulation of dicamba at 0, 0.056, 0.56, and 5.6 g ae $ha^{-1}$

with a 1X rate being 560 g ae $ha^{-1}$ for POST application in DR soybean (Anonymous 2018). All treatments were applied when soybean reached the V3 growth stage.

***Timing of Dicamba Exposure.*** The experiment was arranged as a single-factor randomized complete block design with four replications. Dicamba was applied at 2.8 g ae $ha^{-1}$ at three growth stages prior to glufosinate at the V3 growth stage, in combination with glufosinate at the V3 growth stage, and at three subsequent timings following the glufosinate application. These dicamba application timings included: cotyledon, V1, V2, and V3 stage of soybean growth and 3, 7, and 10 days after the V3 treatment (DATV3). Visible auxin injury to soybean was assessed 21 days after the glufosinate application at the V3 growth stage (Behrens and Leuschen 1979) (Table1).

**Statistical Analysis.** All normally distributed data for both experiments (soybean height, maturity, 100-seed weight, and yield) were analyzed with JMP Pro 14.3 (SAS Institute Inc., Cary, NC) using the Fit Model Function. All injury data were not normally distributed and therefore a beta distribution was assumed. Injury data relative to the nontreated were analyzed with SAS 9.4 using PROC GLIMMIX (Gbur et al. 2012). For the timing of dicamba exposure experiment, contrasts were constructed with SAS under the same assumptions of the beta distribution. For the tank contamination experiment, site-year, herbicide, and dicamba rate were considered fixed effects in the model, while block (replication) was considered a random effect. For the timing of dicamba experiment, site-year and dicamba timing were considered fixed effects and block was considered a random effect. All data were subject to analysis of variance, and means were separated using Fisher's protected LSD ($\alpha = 0.05$).

## RESULTS AND DISCUSSION

**Dicamba Tank-Contamination.** An interaction between site-year and dicamba rate was observed at 21 and 28 DAT, indicating that soybean demonstrated significant foliar response to dicamba rate (Table 2). Greater dicamba injury was observed in 2018 than in 2019, and an increase in dicamba rate likewise resulted in an increase in auxin injury observed for both site-years (Tables 2 and 3). Auxin symptomology elicited by tank contamination rates of dicamba appeared in newer, vegetative trifoliates, ultimately causing leaf cupping, stunting, stacking of nodes, and chlorosis.

In addition to dicamba injury being a function of rate, the combination of contact herbicides with dicamba exacerbated the extent of auxin injury over dicamba alone (Figures 1 and 2). Glufosinate alone, acifluorfen alone, and the mixture of the two herbicides resulted in 7, 18, and 21% injury to soybean, respectively, without the addition of dicamba when averaged over site-year. Postemergence injury is common from labeled applications of PPO herbicides on soybean such as acifluorfen, fomesafen, and lactofen (Aulakh et al. 2016). Labeled rates of glufosinate plus acifluorfen applied as a mixture with dicamba at 5.6 g ae $ha^{-1}$ caused a 20 and 28 percentage point increase in auxin symptomology 21 and 28 DAT, respectively, compared to dicamba treatments alone (Table 4). Similar results were documented with acifluorfen and glufosinate alone when applied with dicamba at 5.6 g ae $ha^{-1}$, suggesting that the presence of contact herbicides may increase the severity of visible auxin symptomology on soybean, which is consistent with research conducted by Kelley et al. (2005). Although antagonism is routinely observed on weed species when contact herbicides are combined with a systemic herbicide such as glufosinate and clethodim or glufosinate and dicamba (Burke et al. 2005; Meyer and Norsworthy 2020), a glufosinate-resistant soybean cultivar will not show contact symptomology

of either glufosinate or acifluorfen to the degree that a weed displays. In the case of a glufosinate-resistant soybean cultivar, as dicamba rate was reduced, the ability to detect an increase in auxin symptomology from the addition of contact herbicides diminished, possibly indicating an interaction of the mixture when dicamba at 5.6 g ae $ha^{-1}$ was in solution (Table 4). Further research is needed to understand the relationship between reduced rates of dicamba and contact herbicides regarding increased auxin symptomology on a glufosinate-resistant soybean.

Despite increased auxin symptomology resulting from mixing dicamba with contact herbicides, penalties to soybean height were only a function of dicamba rate, with soybean height being reduced by 30 percentage points compared to plots treated with acifluorfen, glufosinate, or a combination (52 cm) from contamination rates of dicamba at 5.6 g ae $ha^{-1}$ (Table 5). In general, height reductions were reflective of dicamba rate, but was only reduced at the highest rate of dicamba. However, as seen in other research, the foliar auxin symptomology was more apparent than height reductions (Solomon and Bradley 2014). Previous research has shown that reductions in soybean height often result in yield loss (Weidenhamer et al. 1989). Nevertheless, soybean subjected to reduced rates of dicamba at early growth stages can counteract synthetic auxin injury by producing more pods, potentially justifying why yield loss was not observed (Wax et al. 1969).

Increases in auxin symptomology from the addition of contact herbicides did not translate to a significant maturity delay. Instead, maturity delays were largely a function of dicamba rate, similar to dicamba injury. In 2018, a consistent increase in delay of maturity was observed with dicamba at 0.56 and 5.6 g ae $ha^{-1}$, with a 4- and 7-day delay in maturity relative to plots treated with only acifluorfen, glufosinate, or a combination. Although soybean maturity in 2019 did not follow the same pattern as in 2018, as dicamba rate increased, delays in maturity likewise

increased compared to plots without dicamba contamination (Table 3). Solomon and Bradley (2014) documented a 5- to 8-day maturity delay when dicamba was applied at V3 to soybean at 28 g ae $ha^{-1}$ whereas the same rate caused a 23- to 26-day delay from an R2 application. However, soybean injury, height reductions, and maturity delays resulting from dicamba tank contamination did not adversely impact grain yield, with the only difference being a 350 kg $ha^{-1}$ increase in yield from 2018 to 2019 (data not shown). Behrens and Leuschen (1979) determined that dicamba injury to soybean of approximately 60 to 70% from a single vegetative exposure may impact yield; however, the amount of visible dicamba injury observed in these irrigated experiments was not enough to effectively reduce yield. Another explanation of why yield loss was not observed could be that soybean has been documented to combat abiotic stresses by compensating one yield component for another, with higher rates of dicamba exposure encouraging greater lateral growth (Kelley et al. 2005; Robinson et al. 2013).

**Timing of Dicamba Exposure.** Visible dicamba injury to soybean typically persists for approximately 28 days with injury peaking 14 to 21 days after exposure (Al-Khatib and Peterson 1999). At 21 DAT, an interaction between site-year and dicamba application timing occurred with respect to visible soybean injury from dicamba (Table 6). Classic dicamba injury to soybean in the form of leaf cupping, epinasty, node stacking, or height reduction was variable. However, comparing dicamba injury on soybean by categorizing the incidence of dicamba exposure (dicamba followed by glufosinate, glufosinate followed by dicamba, or dicamba plus glufosinate) as sequential applications relative to a V3 glufosinate application, better characterized the severity of auxin symptomology. Based on contrasts, applications where dicamba preceded glufosinate resulted in less visible injury than treatments where dicamba followed glufosinate (35 and 56%, respectively) (Table 7). A dicamba plus glufosinate mixture

was less injurious as well compared to treatments in which dicamba followed glufosinate (45 and 56%, respectively).

The addition of glufosinate to certain graminicides is known to reduce efficacy in monocot weeds by initiating cell destruction and therefore limiting uptake and translocation of the graminicide (Burke et al. 2005). The antagonistic effects of mixtures of glufosinate and systemic herbicides such as dicamba or 2,4-D vary among species (Merchant et al. 2013). However, metabolism of glufosinate when applied to a glufosinate-resistant soybean cultivar would eliminate the potential for contact symptomology that is responsible for antagonism between contact and systemic herbicides. The compounding stress from metabolism of both glufosinate and dicamba on a glufosinate-resistant soybean cultivar may explain the increased dicamba symptomology associated when a dicamba exposure is followed by a glufosinate application or when applied as a mixture.

Although soybean grain yield improved by 470 kg $ha^{-1}$ (10%) from 2018 to 2019, yield penalties were contingent upon dicamba application timing or exposure (data not shown). Despite multiple treatments injuring soybean beyond 50% relative to the nontreated, dicamba exposure at 7 and 10 DATV3 (V5- to V6-soybean) were the only treatments to reduce yield, possibly due to soybean approaching reproductive development. Overall, soybean is more likely to recover from early-season than late-season dicamba exposure (Slife 1956; Wax et al. 1969; Auch and Arnold 1978; Solomon and Bradley 2014) (Table 8).

**Practical Implications.** Producers have the luxury of planting a multitude of soybean cultivars with various herbicide technologies to combat an ever-evolving weed spectrum, sometimes with resistance to several herbicide sites of action (Anonymous 2020; Heap 2020). Fortunately, many producers are still able to rely on contact herbicides such as glufosinate and fomesafen (where

PPO resistance is not present) to effectively control Palmer amaranth. Unfortunately, adoption of DR soybean for control of problematic broadleaf weeds in some geographies has presented new challenges for producers choosing to plant non-DR technologies. In these geographies, frequent off-target movement of dicamba leads to symptomology on non-DR soybean because of the sensitivity of soybean to extremely low doses (Auch and Arnold 1978; Egan et al. 2014; Jones 2018; Solomon and Bradley 2014).

Findings from the tank contamination experiment indicate that dicamba contamination in a sprayer or off-target movement of the herbicide onto soybean has the potential to interact with POST contact herbicides applied directly to the crop, leading to greater dicamba symptomology on soybean than from a dicamba exposure alone. Although yield was not negatively affected by visible injury from a single exposure of dicamba with or without the addition of contact herbicides, the increased injury could slow canopy development (Priess et al. 2020) and require an additional POST herbicide application to maintain a high level of weed control. Auxin injury may be prolonged, and maturity delayed with multiple exposures to sensitive soybean cultivars, particularly when exposure to dicamba occurs in conjunction with contact herbicides. Based on findings from the timing of dicamba exposure experiment, soybean exposed to dicamba at or following a glufosinate application, will likely elicit greater dicamba symptomology than a prior exposure, with yield reductions occurring resulting from late-season dicamba exposure as seen in other research (Auch and Arnold 1978; Slife 1956; Solomon and Bradley 2014; Wax et al. 1969).

To expand upon the interaction of contact herbicides and dicamba, supplementary tank-contamination research could be beneficial by focusing on the influence of dicamba exposure with flumioxazin, a commonly applied PRE PPO-inhibiting herbicide in soybean. With many

producers in the Midsouth having simultaneous cotton and soybean acres, there is potential for flumioxazin residue to remain in the sprayer when applying POST herbicides in cotton and soybean. Based on this research, the combination of dicamba and flumioxazin could amplify auxin symptomology.

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## TABLES

Table 1. Behrens and Leuschen (1979) soybean dicamba injury index.

| Rating | Description |
|---|---|
| 0 | No effect, plant normal |
| 10 | Slight crinkle of leaflets of terminal leaf |
| 20 | Cupping of terminal leaflets, slight crinkle of leaflets of second leaf, growth rate normal |
| 30 | Leaflets of two terminal leaves cupped, expansion of terminal leaf suppressed slightly |
| 40 | Malformation and growth suppression of two terminal leaves, terminal leaf size less than one-half that of control |
| 50 | No expansion of terminal leaf, second leaf size one-half or less that of control |
| 60 | Slight terminal growth, vigorous, malformed axillary shoot growth developing |
| 70 | Terminal bud dead, substantial, strongly malformed, axillary shoot growth |
| 80 | Limited axillary shoot growth, leaves present at time of treatment chlorotic with slight necrosis |
| 90 | Plant dying, leaves mostly necrotic |
| 100 | Plant dead |

Table 2. The p-values with site-year considered as a fixed-effect from analysis of variance for soybean dicamba injury, height, maturity, 100-seed weight, and yield from 2018 and 2019 in Fayetteville, AR.[a]

| | Auxin injury | | Height | | | |
|---|---|---|---|---|---|---|
| Source | 21 DAT[b] | 28 DAT | 28 DAT | Maturity | 100-seed weight | Yield |
| | ----------Prob. > F---------- | | | | | |
| Site-year | <0.001 | <0.001 | 0.287 | 0.003 | 0.391 | 0.028* |
| Herbicide | 0.003 | <0.001 | 0.466 | 0.537 | 0.402 | 0.206 |
| Site-year*herbicide | 0.770 | 0.822 | 0.140 | 0.713 | 0.404 | 0.816 |
| Dicamba rate | <0.001 | <0.001 | <0.001* | <0.001 | 0.363 | 0.683 |
| Site-year*dicamba rate | <0.001* | <0.001* | 0.240 | <0.001* | 0.349 | 0.966 |
| Herbicide*dicamba rate | <0.001* | <0.001* | 0.747 | 0.969 | 0.432 | 0.478 |
| Site-year*herbicide*dicamba rate | 0.961 | 0.765 | 0.813 | 0.696 | 0.461 | 0.800 |

[a]P-values within columns denoted by asterisks indicate significance. Interactions take precedence over main effects.
[b]Abbreviation: DAT, days after treatment.

Table 3. Interaction of site-year and dicamba rate averaged over contact herbicide on soybean auxin injury and maturity in 2018 and 2019 at Fayetteville, AR.[a]

| Site-year | Dicamba rate | Auxin injury | | Relative maturity[c] |
|---|---|---|---|---|
| | | 21 DAT[b] | 28 DAT | |
| | g ae ha$^{-1}$ | ----------% of nontreated---------- | | days |
| 2018 | 0 | 0 f | 0 f | 0 d |
| | 0.056 | 8 d | 4 e | 1 d |
| | 0.56 | 59 b | 50 c | 4 bc |
| | 5.6 | 71 a | 71 a | 7 ab |
| 2019 | 0 | 0 f | 0 f | 3 cd |
| | 0.056 | 2 e | 2 f | 8 a |
| | 0.56 | 31 c | 25 d | 5 abc |
| | 5.6 | 61 b | 58 b | 5 abc |

[a]Means within a column followed by the same letter are not different according to Fisher's protected LSD (α=0.05).
[b]Abbreviation: DAT, days after treatment.
[c]Data expressed as days delayed relative to the nontreated.

Table 4. Interaction of herbicide and dicamba rate averaged over site-year on soybean auxin injury from 2018 and 2019 at Fayetteville, AR.[a]

| Herbicide | Dicamba rate | Auxin injury | |
|---|---|---|---|
| | | 21 DAT[b] | 28 DAT |
| | g ae ha$^{-1}$ | ----------% of nontreated---------- | |
| None | 0 | 0 g | 0 i |
| | 0.056 | 2 fg | 1 i |
| | 0.56 | 41 d | 32 g |
| | 5.6 | 53 c | 47 d |
| Glufosinate | 0 | 0 g | 0 i |
| | 0.056 | 15 e | 8 h |
| | 0.56 | 46 cd | 42 e |
| | 5.6 | 66 b | 64 c |
| Acifluorfen | 0 | 0 g | 0 i |
| | 0.056 | 2 fg | 2 i |
| | 0.56 | 45 d | 36 f |
| | 5.6 | 70 ab | 69 b |
| Glufosinate+acifluorfen | 0 | 0 g | 0 i |
| | 0.056 | 4 f | 2 i |
| | 0.56 | 47 cd | 35 fg |
| | 5.6 | 73 a | 75 a |

[a]Means within a column followed by the same letter are not different according to Fisher's protected LSD (α=0.05).
[b]Abbreviation: DAT, days after treatment.

Table 5. Soybean height 28 days after treatment averaged over site-year and contact herbicide from 2018 and 2019 at Fayetteville, AR.[a]

| Dicamba rate | Height |
|---|---|
| g ae $ha^{-1}$ | % of nontreated |
| 0 | 97 a |
| 0.056 | 95 a |
| 0.56 | 92 a |
| 5.6 | 70 b |

[a]Means within a column followed by the same letter are not different according to Fisher's protected LSD (α=0.05).

Table 6. The p-values with site-year considered as a fixed-effect from analysis of variance for soybean dicamba injury 21 days after treatment, height 21 days after treatment, maturity, 100-seed weight, and yield from 2018 and 2019 at Fayetteville, AR.[a]

| Source | Auxin injury | Soybean height | Maturity | 100-seed weight | Yield |
|---|---|---|---|---|---|
| | ----------Prob. > F---------- | | | | |
| Site-year | 0.006 | 0.006 | 0.069 | 0.507 | 0.050* |
| Timing | <0.001 | <0.001 | 0.602 | 0.413 | 0.004* |
| Site-year*Timing | <0.001* | <0.001* | 0.078 | 0.844 | 0.467 |

[a]P-values within columns denoted by asterisks indicate significance. Interactions take precedence over main effects.
[b]Abbreviation: DAT, days after treatment.

Table 7. Interaction of site-year and dicamba timing on soybean auxin injury and height 21 days after treatment as well as contrasts for dicamba injury and soybean height when glufosinate was preceded, combined, or followed by dicamba exposure from 2018 and 2019 in Fayetteville, AR.[a]

| Site-year | Timing | Auxin injury | Soybean height |
|---|---|---|---|
| | | ----------% of nontreated---------- | |
| 2018 | VC | 28 d | 90 ab |
| | V1 | 28 d | 99 a |
| | V2 | 50 c | 87 abc |
| | V3 | 55 bc | 70 e |
| | 3 DATV3[b] | 60 abc | 72 de |
| | 7 DATV3 | 63 ab | 74 cde |
| | 10 DATV3 | 55 bc | 86 abcd |
| 2019 | VC | 14 e | 78 bcde |
| | V1 | 29 d | 84 bcde |
| | V2 | 61 ab | 48 f |
| | V3 | 35 d | 79 bcde |
| | 3 DATV3 | 26 d | 87 abc |
| | 7 DATV3 | 61 ab | 75 cde |
| | 10 DATV3 | 70 a | 73 cde |
| Contrast[c] | | | |
| D fb G[d] vs. G fb D | | 35 vs. 56*** | 81 vs. 78 NS |
| D fb G vs. D + G | | 35 vs. 45*** | 81 vs. 75 NS |
| D + G vs. G fb D | | 45 vs. 56** | 75 vs. 78 NS |

[a]Means within a column followed by the same letter are not different according to Fisher's protected LSD (α=0.05).
[b]Abbreviation: DATV3, time of dicamba exposure in days after glufosinate applied at the V3 stage of soybean days after treatment V3
[c]Contrasts: * significant ($P < 0.05$); ** significant ($P < 0.01$); *** significant ($P < 0.001$); NS, nonsignificant ($P \geq 0.05$).
[d]Abbreviations: D, dicamba; fb, followed by; G, glufosinate.

Table 8. Soybean yield averaged over site-year from 2018 and 2019 at Fayetteville, AR.[a]

| Dicamba timing | Yield |
|---|---|
| | % of nontreated |
| VC | 97 ab |
| V1 | 100 a |
| V2 | 116 a |
| V3 | 106 a |
| 3 DATV3 | 99 ab |
| 7 DATV3 | 82 bc |
| 10 DATV3 | 79 c |

[a]Means within a column followed by the same letter are not different according to Fisher's protected LSD (α=0.05).
[b]Abbreviation: DATV3, time of dicamba exposure in days after glufosinate applied at the V3 stage of soybean.

## FIGURES

Figure 1. Synthetic auxin symptomology associated with a dicamba application alone at 5.6 g ae $ha^{-1}$ 28 days following a V3 application to soybean in Fayetteville, AR in 2018.

Figure 2. Synthetic auxin symptomology associated with a contamination rate of 5.6 g ae $ha^{-1}$ dicamba in combination with 656 and 560 g ai $ha^{-1}$ glufosinate and acifluorfen, respectively, 28 days following a V3 application to soybean in Fayetteville, AR in 2018.

# CHAPTER 4

# IMPACT OF DICAMBA ON REPRODUCTIVE DEVELOPMENT IN CORN AND RICE

## ABSTRACT

Application of dicamba products Engenia™ and XtendiMax™ in both cotton and soybean have presented major concerns for off-target movement, primarily to non-dicamba-resistant soybean. Extensive research has been published regarding the effects of sublethal rates of dicamba applied at different growth stages in soybean; however, there is limited research on the impact of dicamba on reproductive corn and rice. To determine the potential consequences of dicamba rate exposure to reproductive corn and rice, experiments were conducted on corn in Fayetteville, Arkansas, and in rice near Stuttgart, Arkansas, in 2018 and 2019. Simulated drift rates of dicamba were applied at 0.56, 5.6, 56, and 560 g ae $ha^{-1}$ to corn and rice, with a 1X rate being 560 g ae $ha^{-1}$. Each dicamba rate was applied at three reproductive growth stages (corn: pre-tassel, tassel, and green silk; rice: late boot, panicle exertion, and anthesis). Visible injury to corn was negligible; however, rice treated with dicamba at 56 and 560 g ae $ha^{-1}$ had noticeably greater injury (3 and 12%, respectively) relative to the nontreated control. For rice, visible injury associated with a 56 and 560 g ae $ha^{-1}$ dicamba application appeared in the form of upright panicles, which reduced panicle weight and seeds per panicle, ultimately decreasing yields by 2,120 and 4,140 kg $ha^{-1}$. A yield loss in corn could be elicited only when dicamba was applied at 560 g ae $ha^{-1}$ during pre-tassel despite dicamba at 560 g ae $ha^{-1}$ reducing both seeds per row and total seeds per ear. With severe consequences being observed only at high dicamba concentrations, the threat of off-target movement to corn and rice is far less severe than those in soybean.

**Nomenclature:** Dicamba; corn, *Zea mays* L.; rice, *Oryza sativa* L.; soybean, *Glycine max* (L.) Merr.

**Keywords:** Dicamba drift, reproductive corn, reproductive rice

## INTRODUCTION

Plant growth regulators or synthetic auxin herbicides such as 2,4-D and methyl-4-chlorophenoxyacetic acid were introduced globally to the market following World War II, which revolutionized modern agriculture. Synthetic auxin herbicides are typically selective against dicot weeds, which allows them to be utilized in cereal crops such as wheat (*Triticum aestivum* L.) and corn. Since the introduction of synthetic auxin herbicides to the market in the 1940s, various chemical families of auxin herbicides have been developed that offer greater selectivity. Families of synthetic auxins include: phenoxycarboxylic acids, benzoic acids, pyridinecarboxylic acids, aromatic carboxymethyl derivatives, and quinolinecarboxylic acids. Synthetic auxin families differ in selectivity due to differences in metabolism to non-phytotoxic molecules and target-site sensitivity between monocots and dicots (Cobb 1992; Sterling and Hall 1997; Grossmann 2003). In order to be effective, synthetic auxin herbicides need a strong negative charge on the carboxyl group of the dissociated molecule that is separated from a weaker positive charge on the aromatic ring (Farrimond 1978). Ultimately, activity of synthetic auxins depends upon sensitivity of plant tissue, physiological stage, and species (Grossmann 2009).

For over 60 years dicamba has been successfully used in corn for the control of broadleaf weeds. Although dicamba is labeled for in-crop use in corn, injury to the crop may be observed if POST applications are made beyond the labeled growth stage, specifically as reproductive development is initiated (Mingxia et al. 2011). Injury to corn has been observed with several synthetic auxin herbicides alongside dicamba, such as 2,4-D and fluroxypyr (Vettakkorumakankav et al. 2002). Adverse consequences from dicamba applied to corn plants at 1, 2, or 4 kg $ha^{-1}$ could be seen at the V3 growth stage, with injury primarily a function of various degrees of lodging 5 days after treatment, with the greatest lodging occurring at the

highest dicamba rate. Corn plants treated with dicamba at 4 kg $ha^{-1}$ saw a reduction in brace and main root strength; however, fluroxypyr disintegrated all roots at the same rate, suggesting labeled synthetic auxin herbicides have potential to cause an unfavorable physiological response at a given rate and growth stage.

Propanil is a photosystem II-inhibiting herbicide commonly used pre-flood in rice for control of grass and broadleaf weed species (Smith and Hill 1990). Prior to the onset of propanil resistance, the foundation of weed control programs in rice generally consisted of sequential postemergence (POST) applications of propanil. Due to the success of propanil and a widespread adoption on rice acres, propanil-resistant biotypes of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] were able to evolve (Baltazar and Smith 1994). The evolution of barnyardgrass resistance coupled with the commercialization of new chemical control options, ultimately led to a decrease in propanil usage.

In 1992, quinclorac was introduced as a chemical option to control broadleaf weeds and propanil-resistant barnyardgrass in rice (Talbert and Burgos 2007). Sharing the same site of action as dicamba and 2,4-D, quinclorac is translocated throughout the plant both acropetally and basipetally, with the youngest leaves acting as sinks (Grossmann 1998; Lamoureux and Rusness 1995). When quinclorac is applied in combination with other rice herbicides or as a stand-alone product, adequate control of problematic weeds could be achieved. Quinclorac has flexibility to be applied pre- and post-flood, however; the label is lenient with respect to salvage applications and is vague regarding a cutoff depending upon rice growth stage (Anonymous 2010). Bond and Walker (2012) found that grain yield was reduced in certain cultivars when rice was treated with quinclorac at panicle initiation (PI) compared to rice treated at an earlier growth stage, determining rice was more likely to be injured from delayed quinclorac applications. Contrary to

grain yield, delays in maturity were affected greater by early post-flood applications compared to later post-flood applications.

Similar to quinclorac, 2,4-D is known to cause injury to rice and is also dependent on application timing. Injury symptoms from 2,4-D include: leaf rolling, erect panicle habit, failure of heads to emerge, reduction in kernels per head, and a delay of maturity, which was ultimately a function of growth stage (Shaw et al. 1955). Data collected from a series of greenhouse and field trials over a three-year period suggested maximized injury from 2,4-D with applications at pre-tillering, boot, and panicle emergence stages of development, contrary to research conducted by Anderson (1996), suggesting greater injury may be observed in younger plants and decrease as plants reach maturity. Injury occurring from 2,4-D applied during pre-tillering, boot, and panicle emergence resulted in varying degrees of yield loss. However, 2,4-D applications made from tillering until boot caused little to no visible injury symptomology (Kaufman 1953).

Because dicamba does not have a label for use in rice, little is known about the extent of injury in comparison to 2,4-D despite sharing a similar chemistry. Unlike synthetic auxin injury documented on rice from untimely applications of 2,4-D, little injury has been observed with dicamba applied to vegetatively active rice even at higher concentrations. However, dicamba applied at or exceeding 56 g ae $ha^{-1}$ during flowering caused noticeable reductions in flag leaf extension, but minimal visible injury at reduced rates. Extensive dicamba symptomology could only be observed at a dicamba concentration of 280 g ae $ha^{-1}$ (Davis et al. 2018).

Excluding 2,4-D, minimal research has been conducted evaluating the impact synthetic auxin herbicides have on corn and rice, specifically addressing potential consequences of dicamba exposure during reproductive development when many in-crop dicamba applications would occur. With ongoing issues regarding the off-target movement of dicamba in the

Midsouthern United States and its impact on sensitive species, assessing the risk of dicamba exposure on traditionally tolerant cereal crops past label restrictions may provide additional insight by any physiological abnormality or reduction in performance. To determine the risk dicamba exposure poses during corn and rice reproductive development, field experiments were conducted in 2018 and 2019.

## MATERIALS AND METHODS

**Common Methodology.** Corn and rice experiments were arranged as a two-factor factorial randomized complete block design with four replications. Factors consisted of four levels of dicamba exposure using the Engenia (BASF Corporation, Research Triangle Park, NC, 27709) formulation (0.56, 5.6, 56 and 560 g ae $ha^{-1}$) and three reproductive growth stages at time of application, which differed by crop. All herbicide applications were made with a $CO_2$-pressurized backpack sprayer calibrated to deliver 140 L $ha^{-1}$ at 4.8 km $hr^{-1}$ equipped with AIXR 110015 flat fan nozzles (Teejet Technologies, Springfield, IL, 62703). Visible injury ratings were assessed 7, 14, 21, and 28 days after treatment (DAT) on a scale of 0 to 100%, with 0 representing no injury and 100 representing plant death. Grain yield was collected for each trial at harvest maturity.

**Corn Field Experiment.** Field experiments were conducted at the Milo J. Schult Agricultural Research and Education Center in Fayetteville, AR, on a Leaf silt loam (Fine, mixed, active, thermic, Typic, Albaquults) with 34% sand, 53% silt, 13% clay, and 1.5% organic matter with a pH of 6.2 in 2018, and a Captina silt loam (Fine-silty, siliceous, active, mesic, Typic Fragiudults) composed of 14% sand, 76% silt, 10% clay, and 4.5% organic matter with a pH of 6.5 in 2019. Prior to planting, the field was prepared with a disk followed by a hipper, which formed 91-cm-wide beds for planting. Once prepared, a 111-day maturing glufosinate and glyphosate-resistant

corn variety (Pioneer 1197YHR) (Pioneer Incorporated, Johnston, IA 50131) was planted at 79,040 seeds $ha^{-1}$ on April 12, 2018, and April 10, 2019, into 1.8-m-wide (two rows) by 7.6-m-long plots with 1.5-m alleys. To maintain trial as weed free, all plots received a PRE application of atrazine at a rate of 1,682 g ai $ha^{-1}$ followed by a POST application of atrazine at 1,121 g ai $ha^{-1}$ at V2-3. Additional POST applications of both glyphosate (Bayer Corporation, Whippany, NJ 07981) and glufosinate (BASF Corporation, Research Triangle Park, NC 27709) were made at 1,542 g ae $ha^{-1}$ and 656 g ai $ha^{-1}$ respectively, throughout the season as needed. Trials were irrigated with over-head irrigation in 2018 and furrow-irrigation in 2019 to simulate standard production practices and create a high yielding environment. The test site both years was fertilized based on soil test recommendations for corn (Espinoza and Ross 2008). In 2018, dicamba was applied at pre-tassel, tassel, and green silk, and growing degree units (GDU) were recorded for each application. In 2019, all applications were made based upon the recorded GDUs (950, pre-tassel; 1135, tassel; 1400, green silk) from the previous year in order for treatments to be received at the precise time.

At harvest maturity, seeds per row and number of rows per ear of five ears were collected from each plot and were counted to assess potential treatment effects. Both rows of each plot were harvested using a small-plot combine (ALMACO, Nevada, IA 50201), and grain yield was determined by adjusting the harvested weights to 13% moisture. Additionally, a grain subsample was collected from the combine during harvest and later used to determine 100-seed weight.

**Rice Field Experiment.** Field experiments were conducted at the Rice Research and Extension Center near Stuttgart, AR, on a Dewitt silt loam (Fine, smectic, thermic, Typic Albaqualfs) made up of 27% sand, 54% silt, 19% clay, and 1.8% organic matter with a pH of 6.9 in 2018 and 2019. Prior to planting, each field was subjected to conventional tillage to prepare the seedbed. On

April 19th in 2018 and April 2nd in 2019, a long-grain, inbred imidazolinone-tolerant cultivar (Clearfield 153) (BASF Corporation, Research Triangle Park, NC, 27709) was drilled into plots 1.8 m wide by 5.2 m long with 1-m alleys. Experiments were kept weed-free with a PRE application of clomazone (FMC Corporation, Philadelphia, PA 19104) at 263 g ai $ha^{-1}$ followed by POST applications of herbicide according to weed species present. All plots were irrigated with an adequate flood maintained from the 5-leaf growth stage until harvest maturity, and soil fertility was addressed according to the Arkansas Rice Production Handbook (Roberts et al. 2016). Herbicide applications were made at three separate growth stages: late boot, panicle exertion, and anthesis.

Along with visible injury, aerial images were taken 2 weeks prior to harvest. At physiological maturity, five panicles from each plot were harvested to measure the weight of each panicle and total seed per panicle. At harvest, a 1.5-m-wide swath out of the 1.8-m-wide plot was harvested using a small-plot combine, and grain yield was determined by adjusting the harvested weights to 12% moisture. Additionally, a grain subsample was collected from the combine during harvest and was later used to determine 100-seed weight.

**Statistical Analysis.** All normally distributed data for corn and rice experiments were analyzed with JMP Pro 14.3 (SAS Institute Inc., Cary, NC) using the Fit Model Function to produce analysis of variance tables to determine significance of fixed effects. Once significance was determined for all assessments, Dunnett's procedure ($\alpha = 0.05$) was used to distinguish if treatments differed from the nontreated control. All injury data were not normal and assumed a beta distribution. Injury data were subjected to analysis of variance and analyzed with SAS 9.4 using PROC GLIMMIX and compared relative to the nontreated control (Gbur et al. 2012). For

each model, replication was nested within year and considered as a random effect. Means were separated using Fisher's protected LSD ($\alpha = 0.05$).

## RESULTS AND DISCUSSION

**Corn Experiment.** Visible injury to corn caused by dicamba during early reproductive growth stages was negligible at all rating dates and therefore was not subjected to statistical analysis (Table 1). Instead, injury of corn induced by dicamba was assessed by analyzing the total number of rows per ear and seeds per row of each ear, seeds per ear, and grain yield. Often, visible injury to corn caused by dicamba occurs as a result of abnormal weather conditions, poorly timed applications, or misapplications (Vettakkorumakankav et al. 2002). Greater visible injury to corn is more likely to occur when dicamba is applied PRE and generally requires lower dicamba rates to express symptomology as opposed to higher rates when corn is more mature (Mingxia et al. 2011), possibly explaining the absence of dicamba symptomology from later-applied dicamba.

Rate of dicamba was the only factor to directly influence the number of corn seeds per row (total number of seeds from bottom of ear to the top of the ear of corn) (Tables 1 and 2). A decrease in the number of corn seeds per row could only be detected when dicamba was applied at 560 g ae $ha^{-1}$, a total reduction of 2 seeds per row or a 7% decline in comparison to the nontreated control. Applications of 2,4-D or dicamba made during the tasseling or dough stage of corn may result in lack of kernel set or grain development, attributing dicamba injury to the documented reduction in seeds per row (Gunsolus and Curran 1998). Dicamba rates less than 560 g ae $ha^{-1}$ did not affect seeds per row relative to the nontreated control (Table 2). Conversely, the number of seeds per circumference (total number of seeds located on the

perimeter of a corn ear cross-section) and seeds per ear were a function of both dicamba rate and growth stage (Table 3).

Although the number of rows per ear can only be an even number, two treatments means were odd numbers (Table 3). The pre-tassel growth stage had two numerical decreases in rows per ear from dicamba at 0.56 and 560 g ae $ha^{-1}$, which resulted in 15 seeds compared to the nontreated control (16). The only treatment to increase the number of rows per ear was dicamba at 0.56 g ae $ha^{-1}$ applied at green silk, although an increase in one row per ear had no impact on yield. All other corn growth stages and dicamba rates had a comparable number of rows per ear.

The total number of seeds per ear of corn was calculated by multiplying the total number of seeds per row by the total number of rows per ear. The total number of seeds per ear was adversely affected only when treated with dicamba at 560 g ae $ha^{-1}$ at the pre-tassel growth stage, which resulted in an 86 seed differential compared to the nontreated control (454) (Table 2). Dicamba applied at 560 g ae $ha^{-1}$ during the pre-tassel reproductive growth stage reduced yield by approximately 2,880 kg $ha^{-1}$ or 32% compared to yield of the nontreated control, which was influenced by the reduction of seeds per row subsequently leading to a reduction in seeds per ear (Table 3). Decreases in yield at the pre-tassel growth stage would suggest that a labeled rate of dicamba (560 g ae $ha^{-1}$) interferes with corn pollination or viability, reducing the potential for additional kernels to be produced. Kernel number is at its greatest potential just before R1, which is ultimately dictated by pollination of the kernel ovule (Lauer 2015). Commonly-applied corn herbicides such as glyphosate can reduce pollen viability if applied beyond V4 in glyphosate-resistant corn as tassel initiation can begin as early as two weeks after emergence (Thomas et al. 2004). The tasseling and green silk growth stage may be less sensitive to dicamba applications if majority of pollination has occurred; however, the cross-pollinating nature of corn

may also be a contributor for the lack of yield loss from applications of dicamba at 560 g ae $ha^{-1}$ during those later reproductive growth stages. Pollen viability may have been impacted in the two treated center rows of corn, but still received viable pollen from the two nontreated rows on the outside of the treated area, thus maintaining yield.

**Rice Experiment.** Applications of synthetic auxin herbicides such as 2,4-D and triclopyr in rice are restricted following panicle initiation (Anonymous 2019, 2008) as plant growth stage can influence susceptibility to herbicides (Ross and Lembi 1998; Bond and Walker 2012). Specifically, generally tolerant crops such as rice may present synthetic auxin symptomology if an application exceeds a physiological cutoff, with seedling stages typically displaying less tolerance over mature plants due to a period of accelerated growth (Anderson 1996; Radosevich et al. 1997). Overall, visible injury to rice from dicamba was minimal, ranging from 0 to 12% when exposure occurred from late boot through anthesis, indicating that visible injury was purely a function of dicamba rate (Tables 4 and 5). Visible injury ratings were based on biomass reduction, visibility of flag leaves, and habit of rice panicles. At high rates of dicamba (56 and 560 g ae $ha^{-1}$), injury was substantially greater than the nontreated control, primarily from reduced visibility of the flag leaf (Figures 1 and 2) and malformed panicles. Panicles treated with 56 and 560 g ae $ha^{-1}$ were noticeably more upright, which may be due to a lack of grain-fill. Findings were consistent with Davis et al. (2018), with visible injury appearing less severe than the penalties to yield and other components. Because of the reduction in flag leaf visibility, dicamba injury visually appeared to increase yield as more panicles were evident; however, canopy coverage analysis did not capture the reductions in canopy formation potentially due to poor image quality

Dicamba rate and rice growth stage played a significant role regarding relative maturity, which was determined when 50% of the panicles within a plot were present (Table 4). Maturity was hastened approximately 2 and 3 days when rice was treated with dicamba at 56 and 560 g ae $ha^{-1}$ dicamba, respectively, at late boot and panicle exertion stages (Figures 1 and 2). However, rice treated with dicamba at 56 g ae $ha^{-1}$ during anthesis did not accelerate maturity (Table 6). Hastening of harvest maturity could potentially benefit Arkansas rice producers as tropical storm systems are common during the harvest months of August and September.

Dicamba injury resulting in upright panicle formation was likely a consequence of reduced panicle weight and number of seeds per panicle. Comparable to visible injury, the only treatments demonstrating adverse effects to rice panicle weight and number of seeds per panicle were dicamba at 56 and 560 g ae $ha^{-1}$, a 20 and 40% reduction in panicle weight and seeds per panicle relative to the nontreated control of 3.45 g and 145 seeds, and both treatments reduced rice yield (Table 5). Yields were reduced by 2,120 and 4,140 kg $ha^{-1}$ compared to the nontreated control (9,700 kg $ha^{-1}$) (Table 5). Bond and walker (2012) reported that rice growth stage at application of quinclorac did not affect rice yield. However, quinclorac was applied only at early reproductive development, whereas dicamba in these experiments was applied at advanced reproductive stages.

**Practical Implications.** Because of a broad range of planting dates of soybean and the potential need for late-season in-crop dicamba applications, the probability for off-target movement of dicamba onto corn and rice during reproductive development is high. However, the overall risk associated with late-season dicamba exposure is low simply because drift-rate concentrations are not enough to elicit an adverse physiological response during corn and rice development or negatively impact grain yield. Ultimately, findings from this research lead to the conclusion that

off-target movement or tank-contamination of dicamba could not be responsible for visible injury or reductions in yield unless a misapplication were to occur. Conversely, because hastening of rice maturity was observed when high rates of dicamba were applied, plant growth regulators may provide utility in the future by accelerating rice maturity in geographies where rice is produced as a staple crop.

Although this research concluded that late-season dicamba injury to corn was negligible, additional studies evaluating the effects of corn pollination could be conducted to confirm that the risk of off-target movement of dicamba on reproductive corn is minimal. For the continuation of this research, corn plants serving as a border in the treated area should be de-tasseled or treated with the center two rows. If the border rows are not de-tasseled or treated with herbicide, pollen from the border rows may influence pollination in the two center rows. Additionally, pollen viability could be evaluated in corn and rice to determine if late-season dicamba exposure causes pollen sterility.

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## TABLES

Table 1. The p-values from analysis of variance for corn injury, rows per ear, seeds per row, seeds per ear, 100-seed weight, and yield from 2018 and 2019 in Fayetteville, AR.[a]

| Source | Injury | Rows per ear | Seeds per row | Seeds per ear | 100-seed weight | Yield |
|---|---|---|---|---|---|---|
| | ----------Prob. > F---------- | | | | | |
| Dicamba rate | | 0.5943 | 0.0024* | 0.0056 | 0.4349 | 0.0010 |
| Application growth stage | | 0.0573 | 0.1284 | 0.0419 | 0.4818 | 0.2697 |
| Dicamba rate*growth stage | | 0.0086* | 0.2198 | 0.0461* | 0.2263 | 0.0434* |

[a]P-values within columns denoted by asterisks indicate significance.

Table 2. Combined corn seeds per row of ear (post-harvest) following four rates of dicamba averaged over growth stage from 2018 and 2019 in Fayetteville, AR.[ab]

| Dicamba rate | Seeds per row |
|---|---|
| g ae $ha^{-1}$ | # |
| 0 | 29 |
| 0.56 | 29 |
| 5.6 | 29 |
| 56 | 29 |
| 560 | 27* |

[a]Means denoted by asterisks within a column are different than the nontreated control according to $\alpha$=0.05 when using Dunnett's procedure.
[b]Growth stages: Late boot, panicle exertion, and anthesis.

Table 3. Interaction of dicamba rate and growth stage to corn on rows per ear and yield in Fayetteville, AR, from 2018 and 2019.[a]

| | | Yield components | | |
|---|---|---|---|---|
| Growth stage | Rate | Rows per ear | Seeds per ear | Yield |
| | g ae ha$^{-1}$ | ----------#---------- | | kg ha$^{-1}$ |
| Pre-tassel | 0 | 16 | 454 | 11,130 |
| | 0.56 | 15 | 443 | 10,690 |
| | 5.6 | 16 | 450 | 11,570 |
| | 56 | 16 | 481 | 10,750 |
| | 560 | 15 | 368* | 7,540* |
| Tassel | 0 | 16 | 454 | 11,130 |
| | 0.56 | 16 | 466 | 11,500 |
| | 5.6 | 16 | 469 | 11,000 |
| | 56 | 16 | 457 | 10,250 |
| | 560 | 16 | 455 | 10,500 |
| Green silk | 0 | 16 | 454 | 11,130 |
| | 0.56 | 17* | 477 | 10,810 |
| | 5.6 | 16 | 481 | 11,070 |
| | 56 | 16 | 458 | 10,560 |
| | 560 | 16 | 442 | 10,060 |

[a]Means denoted by asterisks within a column are different than the nontreated control with respect to growth stage according to $\alpha$=0.05 when using Dunnett's procedure.

Table 4. The p-values from ANOVA for rice injury (3 weeks after herbicide application), canopy coverage, panicle weight, 100-seed weight, seeds per panicle, 50% heading, and yield from 2018 and 2019 near Stuttgart, AR.[a]

| Source | Injury | Canopy coverage | Panicle weight | 100-seed weight | Seeds per panicle | 50% heading | Yield |
|---|---|---|---|---|---|---|---|
| | ----------Prob. > F---------- | | | | | | |
| Dicamba rate | <.0001* | 0.9998 | <.0001* | 0.3390 | <.0001* | <.0001 | <.0001* |
| Growth stage | 0.5406 | 0.1760 | 0.5304 | 0.5121 | 0.7483 | 0.4967 | 0.3816 |
| Dicamba rate*growth stage | 0.2618 | 0.2894 | 0.1067 | 0.8204 | 0.1285 | 0.0283* | 0.8000 |

[a]P-values within columns denoted by asterisks indicate significance.

Table 5. Combined injury (3 weeks after herbicide application), canopy coverage (pre-harvest) panicle weight (post-harvest), 100-seed weight (post-harvest), seeds per panicle (post-harvest), and grain yield of rice following four rates of dicamba averaged over growth stage from 2018 and 2019 near Stuttgart, AR.[ab]

| Dicamba rate | Injury | Canopy coverage | Yield components | | | Yield |
|---|---|---|---|---|---|---|
| | | | Panicle weight | 100-seed weight | Seeds per panicle | |
| g ae ha$^{-1}$ | --% of nontreated-- | | ----------g---------- | | # | kg ha$^{-1}$ |
| 0 | 0 | 90 | 3.45 | 2.39 | 145 | 9,700 |
| 0.56 | 0 c | 91 | 3.58 | 2.42 | 148 | 9,950 |
| 5.6 | 0 c | 92 | 3.50 | 2.37 | 148 | 9,950 |
| 56 | 3 b | 92 | 2.77* | 2.41 | 115* | 7,580* |
| 560 | 12 a | 92 | 2.13* | 2.32 | 92* | 5,560* |

[a]Means denoted by asterisks within a column are different than the nontreated control according to α=0.05 when using Dunnett's procedure.
[b]Growth stages: Late boot, panicle exertion, and anthesis.

Table 6. Interaction of dicamba rate and growth stage on rice maturity (50% heading) near Stuttgart, AR from 2018 and 2019.[a]

| Growth stage | Rate | Maturity[b] |
|---|---|---|
| | g ae $ha^{-1}$ | days |
| Late boot | 0.56 | 1 a |
| | 5.6 | <1 ab |
| | 56 | -2 c |
| | 560 | -3 c |
| Panicle exertion | 0.56 | <1 b |
| | 5.6 | <1 ab |
| | 56 | -2 c |
| | 560 | -3 c |
| Anthesis | 0.56 | <1 ab |
| | 5.6 | <1 b |
| | 56 | -1 b |
| | 560 | -3 c |

[a]Means within a column followed by the same lowercase letter are not different according to Fisher's protected LSD ($\alpha$=0.05)
[b]Maturity measured in days relative to the nontreated control

## FIGURES

Figure 1. Nontreated control 21 days following late-boot applications near Stuttgart, AR in 2019.

Figure 2. Synthetic auxin symptomology on rice causing erect panicles, reduced visibility of flag leaf, and hastening of maturity 21 days following a late-boot application of dicamba at 560 g ae $ha^{-1}$ near Stuttgart, AR in 2019.

## GENERAL CONCLUSIONS

Off-target movement of dicamba caused by physical or vapor drift is a challenge for non-DR soybean producers in the United States, specifically for producers in the Midsouth. Frequent off-target exposures of dicamba leads to symptomology on non-DR soybean due to sensitivity of the crop to extremely low doses of the herbicide. Not only does off-target movement of dicamba have the capability to damage sensitive soybean, dicamba may also physiologically impact cereal crops during key yield determining growth stages without necessarily presenting auxin symptomology.

The potential for non-DR soybean producers to experience an increase in grain yield from off-target dicamba exposure is unlikely and is not evident with any dicamba rate assessed in this research. Dicamba rates applied in the hormetic response study are minimal enough to pose little potential to reduce yield components or yield following a single reproductive exposure. Based on a single extremely low-dose dicamba off-target or tank-contamination event where minor auxin symptomology is present, there is no indication that dicamba increases grain yield.

When assessing a low-dose dicamba exposure from a tank-contamination scenario, dicamba injury to sensitive soybean may impede canopy development, resulting in additional postemergence herbicide applications to maintain a high level of weed control. Auxin injury may persist for extended periods and producers may observe delays in maturity especially with multiple dicamba exposures to sensitive soybean cultivars, which is likely. Based on this research, auxin injury and maturity delays may be intensified if dicamba exposure occurs in the presence of contact herbicides. Despite the presence of contact herbicides, early-season low-dose dicamba exposure should have little impact on yield with a single exposure. Additionally,

soybean exposed to dicamba at or following a glufosinate application, will likely have more dicamba symptomology than exposure prior to a glufosinate application.

Due to the broad range of planting dates of soybean and the potential need for late-season in-crop dicamba applications to maintain adequate weed control, the likelihood for dicamba to move off-target onto corn and rice during reproductive development is high. Given the potential for late-season dicamba exposure to occur, drift rates are not enough to cause an adverse physiological response during corn and rice development or pose consequences to grain yield. This research concludes that off-target movement via physical or vapor drift, or tank-contamination of dicamba could not be accountable for any visible injury or decreases in corn or rice grain yield unless a misapplication were to occur.